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STREPTOGRAMINS AND METHOD FOR PREPARING SAME BY MUTASYNTHESIS

[001] This is a division of application Serial No. 08/765,907, filed March 20, 1997, which is a 371 of PCT/FR95/00889, filed July 4, 1995, all of which are incorporated herein by reference.

[002] The present invention relates principally to novel compounds which are related to the group B streptogramins, and to a process for preparing streptogramins by mutasynthesis. It also relates to novel genes which are involved in the biosynthesis of precursors of the group B streptogramins, and to their uses.

[003] The streptogramins form a homogeneous group of antibiotics consisting of an association of two types of chemically different molecules; on the one hand polyunsaturated macrolactones (group A components) and, on the other hand, depsipeptides (group B components). This group comprises numerous antibiotics which are known under different names according to their origin and includes pristinamycins, mikamycins and virginiamycins (Cocito 1979, 1983).

[004] The A and B components have a synergistic antibacterial activity which can amount to 100 times that of the separate components and which, contrary to that of each component, is bactericidal (Cocito 1979). This activity is more particularly effective against Grai-positive bacteria such as Staphylococci and Streptococci (Cocito 1979, Videau 1982). Components A and B inhibit protein synthesis by binding to the 50S subunit of the ribosome (Cocito 1979; Di Giambattista et al., 1989).

[005] While knowledge of the routes by which each of the components is biosynthesized still remains partial to date, earlier studies, presented in Patent Application PCT/FR93/0923, have made it possible to identify several proteins, and the corresponding structural genes, which are involved in the biosynthesis of the two types of component.

[006] Two parts can be distinguished in the process for biosynthesizing group B streptogramins:

[007] 1) Biosynthesis of the precursors, or their analogues, of the macrocycle: 3-hydropicolinic acid, L-2-aminobutyric acid, 4-dimethylamino-L-phenylalanine, L-pipecolic acid and L-phenylglycine.

[008] 2) Formation of the macrocycle from the precursors listed above, from L-threonine and from L-proline, or their analogues, with (a) possible subsequent modification(s) of the peptide N-methylation, epimerisation, hydroxylation and oxidation type.

[009] Patent Application PCT/FR93/0923 relates, in particular, to the enzymes which catalyze incorporation of the precursors into the peptide chain of B streptogramins in the process of elongation, and also to their structural genes. These results have demonstrated the non-ribosomal peptide synthesis character of the type B components.

[010] The present invention relates, more particularly, to novel compounds which are related to group B streptogramins and, more precisely, to novel compounds of the pristinamycin I family (Figures 1 and 2), termed PI below, or of the virginiamycin S family (Figure 3).

[011] The major constituent of the I pristinamycins (PI) is PI_A (Figure 1), which represents approximately 94% of the PI, with the remaining approximately 6% being represented by minor constituents of the depsipeptide (PI_B, to PI_I) whose structures are depicted in Figure 2. PI results essentially from the condensation of amino acids, certain of which are essential for

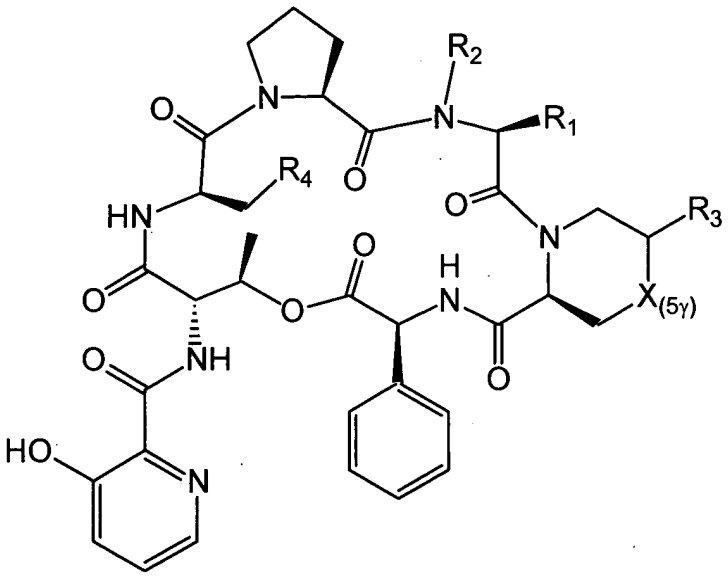
protein synthesis (threonine and proline) and others of which are novel and themselves considered to be secondary metabolites (L-2-aminobutyric acid, 4-dimethylamino-L-phenylalanine (DMPAPA), L-pipecolic acid and L-phenylglycine for PI_A), and also of an aromatic precursor, 3-hydroxypicolinic acid.

[012] The virginiamycin S derivatives result from condensation of the same acids as in the case of PI, apart from 4-DMPAPA, which is replaced by a phenylalanine (see Figure 3).

[013] Production of these different compounds by biosynthesis therefore requires preliminary synthesis, by a producer strain, of the novel precursors identified above.

[014] The present invention results specifically from a novel process for preparing streptogramins which employs, as a strain for producing streptogramins, a microorganism strain which is mutated so as to alter the biosynthesis of the precursors of the group B streptogramins. According to this process, the said mutant strain is cultured in a medium which is supplemented with a novel precursor which is different from the precursor whose biosynthesis is altered. Unexpectedly, this results in the production of novel compounds which are related to the group B streptogramins and which are of value in the therapeutic sphere.

[015] More precisely, the present invention relates to novel compounds which are represented by the general formula I:



I

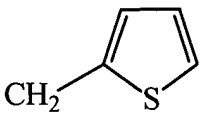
in which:

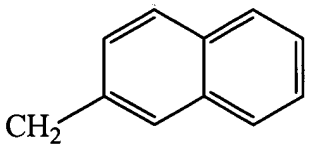
- R_2 and R_4 represent, independently of each other, a hydrogen atom or a methyl group,

- R_3 represents a hydrogen atom or a hydroxyl group,

- X represents a CO, CHOH or CH_2 group, and

- R_1 represents:





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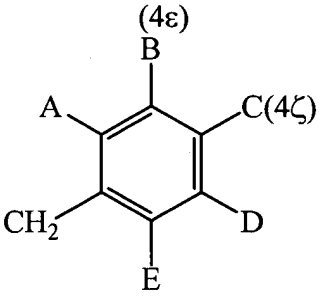

with

[016] - <u>for the meta derivatives:</u>

A, C, D and E representing a hydrogen atom, and

B being able to represent

- a halogen, and preferably a fluorine atom,

- a monoalkylamino or dialkylamino group,

with alkyl preferably representing a methyl or ethyl group,

- an ether group; more particularly an OR group with R being preferably selected from among the methyl, ethyl, trifluoromethyl and allyl groups,

- a thioether group, preferably represented by an alkylthio group with alkyl preferably representing a methyl group,

- a C_1 to C_3 alkyl group, or

- a trihalogenomethyl group, preferably trifluoromethyl

[017] - <u>for the para derivatives</u>

A, B, D and E representing a hydrogen atom, and C being able to represent:

- a halogen,

- an NR_1R_2 group with R_1 and R_2 representing, independently of each other, a group selected from among

- hydrogen,

- a straight-chain or branched C_1 to C_4 alkyl group where, when one of the substituents R_1 or R_2 represents a methyl group, the other necessarily represents an ethyl group,

- an alkyl-cycloalkylmethyl group with a C_3 to C_4 cycloalkyl,

- an optionally substituted C_3 to C_4 cycloalkyl group,

- a straight-chain or branched C_1 to C_4 alkenyl group where, when one of the substituents R_1 or R_2 represents an alkenyl group, the other is different from a methyl group or a C_3 to C_6 cycloalkyl,

- a substituted or unsubstituted N- pyrrolidinyl group,

- an ether group; preferably an OR group with R preferably being selected from among the methyl and ethyl groups, where appropriate substituted by a chlorine atom, or trifluoromethyl and alkenyl groups

- a thioether group, preferably represented by an alkylthio group with alkyl preferably representing a C_1 to C_3 alkyl group,

- an acyl or alkoxycarbonyl group and, more particularly, a COR group with R preferably representing a C_1 to C_3 alkyl group or a C_1 to C_3 alkoxy group,

- a C_1 to C_6 alkyl group which is straight-chain or branched and which is preferably selected from among the methyl, isopropyl and tert-butyl groups,

- an alkylthiomethyl group and, more preferably, a CH_2SR group with R preferably representing a C_1 to C_3 alkyl group,

- an aryl group, preferably a phenyl group, or

- a trihalogenomethyl group, preferably trifluoromethyl

[018] - <u>for the meta-para disubstituted derivatives:</u>

A, D and E representing a hydrogen atom, and

B being able to represent:

- a halogen, preferably a fluorine atom,

- a monoalkylamino or dialkylamino group with alkyl preferably representing a methyl or ethyl group,

- an ether group and preferably an OR group with R preferably selected from among the methyl, ethyl and trifluoromethyl groups,

- a thioether group and preferably alkylthio with alkyl preferably representing an ethyl group, or

- a C_1 to C_3 alkyl group, and

C being able to represent:

- a halogen, preferably a fluorine atom,

- an amino, monoalkylamino or dialkylamino group with alkyl preferably representing a methyl group with the proviso that B is different from a bromine or chlorine atom, or a substituted or unsubstituted allyl group,

- an ether group and preferably an OR group with R preferably selected from among the methyl, ethyl and trifluoromethyl groups,

- a thioether group and preferably an alkylthio group with alkyl preferably representing a methyl group,

- a C_1 to C_6 alkyl group, or

- a trihalogenomethyl group, preferably trifluoromethyl, and

[019] - <u>for the ortho-para disubstituted derivatives:</u>

B, E and D representing a hydrogen atom and A and C a methyl group.

[020] The following may be more particularly mentioned as preferred compounds:

4ζ-methylthio-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-methylthio-de(4ζ-dimethylamino)pristinamycin I_H,

5γ-hydroxy-4ζ-methylthio-de(4ζ-dimethylamino)pristinamycin I_H,

4ζ-methyl-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-methyl-de(4ζ-dimethylamino)pristinamycin I_H,

4ζ-methoxy-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-methoxycarbonyl-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-chloro-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-bromo-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-bromo-de(4ζ-dimethylamino)pristinamycin I_H,

4ζ-iodo-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-iodo-de(4ζ-dimethylamino)pristinamycin I_H,

4ζ-trifluoromethyl-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-trifluoromethyl-de(4ζ-dimethylamino)pristinamycin I_H,

4ζ-tert-butyl-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-isopropyl-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-isopropyl-de(4ζ-dimethylamino)pristinamycin I_E,

4ε-methylamino-de(4ζ-dimethylamino)pristinamycin I_A,

4ε-methoxy-de(4ζ-dimethylamino)pristinamycin I_A,

4ε-methoxy-de(4ζ-dimethylamino)pristinamycin I_H,

4ε-fluoro 4ζ-methyl-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-amino-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-ethylamino-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-diethylamino-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-allylamino-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-diallylamino-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-allylethylamino-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-ethylpropylamino-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-ethylisopropylamino-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-ethylmethylcyclopropylamino-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-(1-pyrrolidinyl)-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-trifluoromethoxy-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-allyloxy-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-ethoxy-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-ethylthio-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-methylthiomethyl-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-(2-chloroethoxy)-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-acetyl-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-ethyl-de(4ζ-dimethylamino)pristinamycin I_A,

4ζ-ethyl-de(4ζ-dimethylamino)pristinamycin I_H,

4ε-dimethylamino-de(4ζ-dimethylamino)pristinamycin I_A

4ε-methylthio-de(4ζ-dimethylamino)pristinamycin I_A,

4ε-ethoxy-de(4ζ-dimethylamino)pristinamycin I_A.

[021] The present invention is also directed towards a process which is particularly useful for preparing the compounds of the general formula I.

[022] More precisely, it relates to a process for preparing streptogramins, characterized in that it employs a streptogramin-producing microorganism strain which possesses at least one genetic modification which affects the biosynthesis of a precursor of the group B streptogramins, and in that the said mutant strain is cultured in a culture medium which is appropriate and which is supplemented with at least one novel precursor which is different from that whose biosynthesis is altered, and in that the said streptogramins are recovered.

[023] The strains which are employed within the scope of the present invention are therefore strains which produce streptogramins and which are mutated. The genetic modification(s) can be located either within one of the genes which is involved in the biosynthesis of the said precursors or outside the coding region, for example in the regions responsible for the expression and/or the transcriptional or post-transcriptional regulation of the said genes, or in a region belonging to the transcript containing the said genes.

[024] According to one particular embodiment of the invention, the mutant strains possess one or more genetic modifications within at least one of their genes which is/are involved in the biosynthesis of the group B streptogramin precursors.

[025] This or these genetic modification(s) alter(s) the expression of the said gene, that is render(s) this gene, and, as the case may be, another of the genes involved in the biosynthesis of the precursors, partially or totally incapable of encoding the natural enzyme which is involved in the biosynthesis of at least one precursor. The inability of the said genes to encode the natural proteins may be manifested either by the production of a protein which is inactive due to structural or conformational modifications, or by the absence of production, or by the production of a protein having an altered enzymatic activity, or else by the production of the natural protein at an attenuated level or in accordance with a desired mode of regulation. The totality of these possible manifestations is expressed by an alteration of, or perhaps a blockage in, the synthesis of at least one of the group B streptogramin precursors.

[026] The genes which are capable of being mutated within the scope of the present invention are preferably the genes which are involved in the biosynthesis of the following precursors: L-2-aminobutyric acid, 4-dimethylamino-L-phenylalanine (DMPAPA), L-pipecolic acid, L-phenylglycine and/or 3-hydroxypicolinic acid (3-HPA).

[027] These genes are more preferably the papA (SEQ ID NO: 14), papM (SEQ ID NO: 16), papB (SEQ ID NO: 4), papC (SEQ ID No. 2), hpaA (SEQ ID NO: 12), snbF (SEQ ID NO: 9), and pipA (SEQ ID NO: 7) genes described below.

[028] The papA and papM genes have already been described in Patent Application PCT/FR93/0923. They are present on the cosmid pIBV2. The papA pap gene appears to correspond to a gene for biosynthesizing 4-amino-L- phenylalanine from chorismate. The 4-amino-L- phenylalanine is then dimethylated by the product of the papM gene, an N-methyltransferase, in order to form 4-dimethylamino-L-phenylalanine, DMPAPA, which is then incorporated into pristinamycin I_A. These two genes are more particularly involved, therefore, in the synthesis of the precursor termed DMPAPA.

[029] The other genes, papB, papC, pipA, snbF and hpaA, have been identified and characterized within the scope of the present invention. They are grouped together with the snbA, papA and papM genes on a chromosomal region of approximately 10 kb (Figure 7).

[030] The sequence homologies demonstrated for the PapB (SEQ ID NO: 5) and PapC (SEQ ID NO: 3) proteins show that these proteins are also involved, jointly with the PapA (SEQ ID NO: 15) and PapM (SEQ ID NO: 17) proteins, in the biosynthesis of the DMPAPA precursor. The two corresponding novel genes, papB and papC, were isolated and identified by subcloning which was carried out using cosmid pIBV2, described in Patent Application PCT/FR93/0923, and a plasmid, pVRC900, which is derived from pIBV2 by means of a HindIII deletion and is also described in Patent Application PCT/FR93/0923.

[031] The comparison of the protein encoded by the papC gene with the protein sequences contained in the Genpro library shows a 27% homology with the region which is involved in the prephenate dehydrogenase activity of the bifunctional TyrA proteins of E. coli

(Hudson and Davidson, 1984) and Erwinia herbicola (EMBL data library, 1991). This region of TyrA catalyses aromatization of the prephenate to form 4-hydroxyphenylpyruvate in the biosynthesis of tyrosine. A similar aromatization, which proceeds from 4-deoxy-4-aminoprephenate and leads to 4-aminophenyl- pyruvate is very probably involved in the synthesis of DMPAPA. It would be catalysed by the PapC protein (SEQ ID No. 2).

[032] PapB possesses a 24 to 30% homology with the region which is involved in the chorismate mutase activity of the TyrA and PheA bifunctional proteins of E. coli, (Hudson and Davidson, 1984) and of the TyrA protein of Erwinia herbicola. This region catalyses isomerization of the chorismate to form prephenate in the biosynthesis of tyrosine and of phenylalanine. The PapB protein (SEQ ID No. 3) is probably involved in a similar isomerization which proceeds from 4-deoxy-4- aminochorismate and leads to 4-deoxy-4-aminoprephenate in the synthesis of DMPAPA.

[033] The pipA, snbF and hpaA genes have been located in the regions which are contained between the snbA gene, which encodes 3-hydroxypicolinic acid AMP ligase and is described in Patent Application PCT/FR93/0923, and the papA or snbR genes. They were located accurately by means of subcloning, which was carried out using the plasmid pVRC900 and the cosmid pIBV2, which are described in Patent Application PCT/FR93/0923.

[034] On comparing the protein encoded by the hpaA gene and the protein sequences contained in the Genpro library, a homology of from 30 to 40% was detected with a group of proteins which are probably involved (Thorson et al., 1993) in the transamination of intermediates in the biosynthesis of various antibiotics (DnrJ, EryCl, TylB, StrS and PrgL). Synthesis of the 3-HPA precursor, which appears to derive from lysine by another route than that of cyclodeamination (see examples 1-2 and 2-1), probably requires a transamination step which

can be catalyzed by the product of this gene termed hpaA (SEQ ID NO: 12). Furthermore, the results of mutating this gene demonstrate unequivocally that it is involved in the synthesis of the 3-HPA precursor.

[035] Comparison of the product encoded by the gene termed pipA with the protein sequences contained in the Genpro library shows a 30% homology with the ornithine cyclodeaminase of Agrobacterium tumefaciens (Schindler et al., 1989). This enzyme is involved in the final step of the catabolism of octopine; it converts L-ornithine into L-proline by means of cyclodeamination. Authors have demonstrated, by means of incorporating labelled lysine, that 4-oxopipecolic acid and 3-hydroxypicolinic acid, which are found both in PI_A and in virginiamycin S1, derived from lysine (Molinero et al., 1989, Reed et al., 1989). Cyclodeamination of lysine, in a similar manner to that described for ornithine, would lead to the formation of pipecolic acid. Taking this hypothesis into account, this product was termed PipA (SEQ ID NO: 7). The results of mutating the pipA gene, presented in the examples below, demonstrate that it is involved solely in the synthesis of pipecolic acid. It is noted, in particular, that this mutation has no effect on the biosynthesis of 3-hydroxypicolinic acid, which is also derived from lysine and of which pipecolic acid could have been a precursor.

[036] Finally, on comparing the product of the gene termed snbF with the protein sequences contained in the Genpro library, a 30 to 40% homology was noted with several hydroxylases of the cytochrome P450 type, which are involved in the biosynthesis of secondary metabolites (Omer et al., 1990; Trower et al., 1992). Several hydroxylations can be envisaged in the biosynthesis of the precursors of pristinamycin I, in particular in the biosynthesis of 3-HPA (hydroxylation of picolinic acid at the 3 position) and of 4-oxopipecolic acid (hydroxylation of pipecolic acid at the 4 position). The corresponding protein was termed SnbF (SEQ ID NO: 9).

[037] The results of mutating the pipA gene, with polar effects on the expression of the snbF gene, demonstrate the involvement of the snbF gene in the hydroxylation of the pipecolic acid residue of group B streptogramins. The expression of the snbF gene is thus altered by the expedient of effecting a genetic modification of the pipA gene.

[038] Preferentially, the genetic modification(s) render(s) the said gene partially or totally incapable of encoding the natural protein.

[039] Genetic modification should be understood to mean, more particularly, any suppression, substitution, deletion, or addition of one or more bases in the gene(s) under consideration. Such modifications may be obtained in vitro (on the isolated DNA) or in situ, for example, by means of genetic engineering techniques, or else by exposing the said microorganisms to a treatment using mutagenic agents. Examples of mutagenic agents which may be cited are physical agents such as high energy rays (X, γ, ultraviolet, etc. rays), or chemical agents which are able to react with different functional groups of the DNA bases, and, for example, akylating agents [ethyl methanesulphonate (EMS), N-methyl-N'-nitro-N-nitrosoguanidine, and N-nitroquinoline-1-oxide (NQO)], bialkylating agents, intercalating agents, etc. Deletion is understood to mean any suppression of a part or all of the gene under consideration. This deletion can, in particular, be of a part of the region encoding the said proteins, and/or of all or part of the promoter region for transcription or translation, or else of the transcript.

[040] The genetic modifications may also be obtained by means of gene disruption, for example using the protocol initially described by Rothstein [Meth. Enzymol. 101 (1983) 202] or, advantageously, by means of double homologous recombination. In this case, the integrity of the coding sequence will preferentially be disrupted in order to permit, if need be, replacement, by

means of homologous recombination, of the wild-type genomic sequence with a non-functional or mutant sequence.

[041] According to another option of the invention, the genetic modifications can consist of placing the gene(s) encoding the said proteins under the control of a regulated promoter.

[042] The mutant microorganism strains according to the present invention may be obtained from any microorganism which produces streptogramins (cf. Table V). According to one particular embodiment of the invention, the mutant strain is a strain which is derived from S. pristinaespiralis and, more particularly, from S. pristinaespiralis SP92.

[043] Mutant strains which are preferred within the scope of the present invention and which may more particularly be mentioned are the strain SP92::pVRC508, which is mutated in the biosynthesis of the DMPAPA precursor by disrupting the papA gene by means of simple crossing over, or else, more preferably, the strain SP212, which is mutated in the biosynthesis of the DMPAPA cursor by disrupting the papA gene by means of double homologous recombination. These strains no longer produce PI unless they are supplemented with the DMPAPA precursor. Unexpectedly, when a novel precursor, which is different from DMPAPA and which is capable, after, in this case, metabolization, of being incorporated by PI synthetase III (SnbD protein which is responsible for incorporating L-proline and DMPAPA residues) is added to the production medium, these two strains then become able to produce novel I pristinamycins or virginiamycins, or else mainly to produce a component which is normally a minor component of PI, in particular PI_B (Figure 2).

[044] Two other mutant strains have been prepared within the scope of the present invention. These are, respectively, the strain SP92pipA::Ωam^R, in which the pipA gene is

disrupted by homologous recombination, and the strain SP92hpaA::Ωam^R, in which the hpaA gene is disrupted. While strain SP92pipA::Ωam^R no longer produces PI under standard fermentation conditions it strongly produces, in the presence of L-pipecolic acid, a component, which was initially a minor component among the B streptogramin components, in which 4-oxopipecolic acid is replaced by L-pipecolic acid. While strain S. pristinaespiralis SP92hpaA::Ωam^R no longer produces PI under standard fermentation conditions, it is able to produce novel group B streptogramins in the presence of novel precursors.

[045] By supplementing the medium for culturing mutant strains according to the invention with at least one novel precursor, it turns out that it is possible to orient biosynthesis either towards novel streptogramins, or towards a minor form of the streptogramins, or else to favour formation of one of the streptogramins.

[046] The precursors which are employed within the scope of the present invention can be derivatives or analogues of amino acids and, more particularly of phenylalanine, as well as organic acids and, in particular, alpha-cetocarboxylic acids and, more particularly, derivatives of phenylpyruvic acid.

[047] Naturally, the novel precursor is such that it complements the alteration or blockage, which is induced in accordance with the invention, within the biosynthesis of one of the natural precursors of the group B streptogramins and leads to the synthesis of streptogramins. According to one particular embodiment of the invention, this novel precursor is selected such that it is related to the precursor whose biosynthesis is altered. Thus, in the specific case of the mutant which is blocked in the biosynthesis of DMPAPA, the novel precursor is preferably a derivative of phenylalanine.

[048] The following may, in particular, be cited as precursors which are suitable for the invention:

[049] Phenylalanine, 4-dimethylaminophenylalanine, 4-methylaminophenylalanine, 4-aminophenylalanine, 4-diethylaminophenylalanine, 4-ethylaminophenylalanine, 4-methylthiophenylalanine, 4-methylphenylalanine, 4-methoxyphenylalanine, 4-trifluoromethoxyphenylalanine, 4-methoxycarbonylphenylalanine, 4-chlorophenylalanine, 4-bromophenylalanine, 4-iodophenylalanine, 4-trifluoromethylphenylalanine, 4-tert-butylphenylalanine, 4-isopropylphenylalanine, 3-methylaminophenylalanine, 3-methoxyphenylalanine, 3-methylthiophenylalanine, 3-fluoro- 4-methylphenylalanine, L-pipecolic acid, 4-tert-butylphenylpyruvic acid, 4-methylaminophenylpyruvic acid, 2-naphthylphenylalanine, 4-fluorophenylalanine, 3-trifluorophenylalanine, 3-ethoxyphenylalanine, 2,4-dimethylphenylalanine, 3,4-dimethylphenylalanine, 3-methylphenylalanine, 4-phenylphenylalanine, 4-butylphenylalanine, 2-thienyl-3-alanine, 3-trifluoromethylphenylalanine, 3-hydroxyphenylalanine, 3-ethylaminophenylalanine, 4-allylaminophenylalanine, 4-diallylaminophenylalanine, 4-allylethylaminophenylalanine, 4-ethylpropylaminophenylalanine, 4-ethylisopropylaminophenylalanine, 4-ethylmethylcyclopropylaminophenylalanine, 4-(1-pyrrolidinyl) phenylalanine, 4-O-allyltyrosine, 4-O-ethyltyrosine, 4-ethylthiophenylalanine, 4-ethylthiomethylphenylalanine, 4-O-(2-chloroethyl)tyrosine, 4-acetylphenylalanine, 4-ethylphenylalanine, 3-dimethylaminophenylalanine, 3-ethoxyphenylalanine, 3-fluoro-4-methylphenylalanine and 4-aminomethylphenylalanine.

[050] Among these precursors, 4-trifluoromethoxyphenylalanine, 3-methylaminophenylalanine, 3-methylthiophenylalanine, 3-fluoro-4-methylphenylalanine, 4-

methylaminophenylpyruvic acid, 3-ethoxyphenylalanine, 4-allylaminophenylalanine, 4-diallylaminophenylalanine, 4-allylethylaminophenylalanine, 4-ethylpropylaminophenylalanine, 4-ethylisopropylaminophenylalanine, 4-ethylmethylcyclopropylaminophenylalanine, 4-(1-pyrrolidinyl) phenylalanine, 4-ethylthiomethylphenylalanine, 4-O-(2-chloroethyl) tyrosine, 3-dimethylaminophenylalanine and 3-ethylaminophenylalanine are novel and were prepared and characterized within the scope of the present invention. They are found to be particularly useful for preparing streptogramins according to the invention.

[051] The claimed process turns out to be particularly adventageous for preparing novel group B streptogramins or else for favoring formation of particular streptogramins. As such, it is particularly useful for preparing PI_B.

[052] The present invention also relates to a nucleotide sequence which is selected from among:

[053] (a) all or part of the genes papC (SEQ ID No. 2), papB (SEQ ID NO: 4), pipA (SEQ ID NO: 7), snbF (SEQ ID NO: 9) and hpaA (SEQ ID NO: 12),

[054] (b) sequences which hybridize with all or part of the (a) genes, and

[055] (c) sequences which are derived from (a) and (b) sequences on account of the degeneracy of the genetic code.

[056] In the particular case of the hybridizing sequences according to (b), these sequences preferably encode a polypeptide which is involved in the biosynthesis of the streptogramins.

[057] Still more preferably, the invention relates to the nucleotide sequences which are represented by the genes papC (SEQ ID No. 2), papB (SEQ ID NO: 4), pipA (SEQ ID NO: 7), snbF (SEQ ID NO: 9), and hpaA (SEQ ID NO: 12).

[058] The invention furthermore relates to any recombinant DNA which encompasses a papC (SEQ ID No. 2), papB (SEQ ID NO: 4), pipA (SEQ ID NO: 7), snbF (SEQ ID NO: 9) or hpaA (SEQ ID NO: 12) gene.

[059] Naturally, the nucleotide sequences defined above can be part of a vector of the expression vector type, which can be an autonomously replicating vector, an integrated vector or a suicide vector. The present invention is also directed to these vectors as well as to any use of a sequence according to the invention or of a corresponding vector for, in particular, preparing metabolites of interest. It furthermore relates to any polypeptide which results from the expression of a claimed sequence.

[060] The present invention also relates to any mutated S. pristinaespiralis strain which possesses at least one genetic modification within one of the papC (SEQ ID No. 2), papB (SEQ ID NO: 4), pipA (SEQ ID NO: 7), snbF (SEQ ID NO: 9) and hpaA (SEQ ID NO: 12) genes, and, more preferably, to strains SP92pipA::Ωam^R and SP92hpaA::Ωam^R, as well as any *S. pristinaespiralis* strain, such as SP212, which possesses a genetic modification which consists of a disruption of the papA gene by means of double homologous recombination.

[061] Combinations of a component of the group A streptogramins and of a compound of the general formula I, according to the invention, constitute compositions which are particularly advantageous in the therapeutic sphere. They are employed, in particular, for treating ailments which are due to Gram-positive bacteria (of the genera Staphylococci, Streptococci, Pneumococci and Enterococci) and Gram-negative bacteria (of the genera Haemophilus, Gonococci, Meningococci). Thus, the compounds according to the invention have a synergistic effect on the antibacterial action of pristinamycin IIB on Staphylococcus aureus

IP8203 in mice in vivo, at oral doses which are principally between 30 mg/kg and 100 mg/kg, when they are combined in PI/PII proportions of the order of 30/70.

[062] The present invention extends to any pharmaceutical composition which contains at least one compound of the general formula I which is or is not combined with a group A streptogramin.

[063] The examples appearing below are presented by way of illustrating the present invention and do not limit it.

[064] LIST OF FIGURES.

Figure 1: Structure of pristinamycin I_A.

Figure 2: Structure of the minor components of pristinamycin I.

Figure 3: Other examples of structures of B components of streptogramins.

Figure 4: Depiction of the PstI-XhoI region of 2.9 kb.

Figure 5: Depiction of the XhoI-PstI region of 4.5 kb.

Figure 6: Depiction of the HindIII-BglII region of 1.6 kb.

Figure 7: Depiction of the BglII-XhoI region of approximately 10 kb.

Figure 8: Depiction of plasmid pVRC415.

Figure 9: Depiction of plasmid pVRC420.

Figure 10: Depiction of plasmid pVRC411.

Figure 11: Depiction of plasmid pVRC421.

Figure 12: Depiction of plasmid pVRC414.

Figure 13: Strategy for constructing SP212.

[065] **EXAMPLE 1: Sequencing and identification of genes involved in the biosynthesis of pristinamycin I and its precursors.**

[066] Identification, by means of sequencing, of the genes situated downstream and upstream of the gene which encodes the enzyme PapA and which is described in Patent PCT/FR93/0923, as well as of a gene which is situated downstream of the gene which encodes the enzyme SnbA and which is also described in Patent PCT/FR93/0923.

[067] This example describes how, using cosmid pIBV2, which is described in Patent PCT/FR93/0923 and which contains the structural genes for the enzymes PapA and PapM, which are involved in the synthesis of the 4-dimethylamino-L-phenylalanine (DMPAPA) precursor of pristinamycin I, and the structural gene for the enzyme SnbA, which is responsible for activating the aromatic precursor, 3-hydroxypicolinic acid (3-HPA), of pristinamycin I, it proved possible to identify, by sequencing around these genes and studying the corresponding mutants, other genes which are involved in the biosynthesis of the DMPAPA precursor or in the biosynthesis of other precursors of pristinamycin I.

[068] With this aim in mind, subclonings were carried out using cosmid pIBV2 and plasmid pVRC900, which is derived from pIBV2 by means of a HindIII deletion and which is also described in Patent PCT/FR93/0923.

[069] This example illustrates how the nucleotide sequences of fragments situated downstream and upstream of the papA and snbA genes of S. pristinaespiralis can be obtained.

[070] The techniques for cloning DNA fragments of interest in the M13mp18 and 19 vectors (Messing et al. 1981) are standard techniques for cloning in Escherichia coli and are described in Maniatis et al. (1989).

[071] 1-1 Sequencing and analysis of the region downstream of the papA gene

[072] In order to sequence this region, which is contained between the papA and papM genes, the PstI-PstI fragment of 1.5 kb, the PstI-XhoI fragment of 0.7 kb, and the XhoI-XhoI fragment of 0.7 kb were subcloned into the M13mp18 and M13mp19 vectors proceeding from plasmid pVRC900. The cloning sites were sequenced through by sequencing on double-stranded DNA using plasmids pVRC900 and pVRC409, which are described in Patent PCT/FR93/0923.

[073] The clonings were carried out as follows. Approximately 2 µg of plasmid pVRC900 were cut with restriction enzymes PstI and/or XhoI (New England Biolabs) under the conditions recommended by the supplier. The restriction fragments thus obtained were separated on a 0.8% agarose gel, and the 1.5 kb PstI-PstI, 0.7 kb PstI-XhoI and 0.7 kb XhoI- XhoI fragments of interest were isolated and purified using Geneclean (Bio101, La Jolla, California). For each cloning, approximately 10 ng of M13mp19 and/or M13mp18, cut with PstI and/or XhoI, were ligated to 100 ng of the fragment to be cloned under the conditions described by Maniatis et al. 1989. After transforming the strain TG1 (K12, Δ(*lac-pro*) *supE thi hsd* ΔS F' *tra*D36 *pro*A^+B^+ *lac*I^q *lacZ* Δ M15; Gibson, 1984) and selecting lysis plaques on an LB + X-gal + IPTG medium in accordance with the technique described by Maniatis et al. (1989), the phage carrying the desired fragments were isolated. The different inserts were sequenced by the chain termination reaction using, as the synthesis primer, the universal primer or synthetic oligonucleotides which were complementary to a 20 nucleotide sequence of the insert to be sequenced. The reactions were carried out using fluorescent dideoxynucleotides (PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit-Applied Biosystem) and analysed on a model 373 A Applied Biosystems DNA sequencer. The overlap between these different inserts

was such that it was possible to establish the entire nucleotide sequence between the papA and papM genes (SEQ ID No. 1).

[074] With the aid of this nucleotide sequence, it is possible to determine the open reading frames and thereby identify genes which are involved, in S. pristinaespiralis, in the biosynthesis of PI or its precursors, as well as the polypeptides encoded by these genes.

[075] We looked for the presence of open reading frames within the 2.9 kb PstI-XhoI fragment, which contains the nucleotide sequence between the papA and papM genes, making use of the fact that Streptomyces DNA displays a high percentage of G and C bases as well as a strong bias in the use of codons which make up the coding frames (Bibb et al. 1984). The method of Staden and McLachlan (1982) makes it possible to calculate the probability of coding frames in terms of the codon usage of Streptomyces genes which have already been sequenced and which are assembled in a data file which contains 19673 codons and which was obtained using the BISANCE (Dessen et al. 1990) computer server.

[076] Using this method, it was possible to characterize four highly probable open reading frames within the 2.9 kb PstI-XhoI fragment, which reading frames are depicted in the table below (TABLE I). They are designated frames 1 to 4 according to their position starting from the PstI site. The length of each reading frame in bases, has been indicated, as has its position within the fragment (the PstI site being situated at position 1); the number of amino acids in the encoded polypeptide has also been indicated for open reading frames 2 and 3. Frames 1, 3 and 4 are encoded by the same strand, while frame 2 is encoded by the complementary strand (Figure 4). Frames 1 and 4 correspond, respectively, to the C-terminal region of the PapA protein and to the N-terminal region of the PapM protein, which proteins were previously identified and described in Patent PCT/FR93/00923.

Frame number and/or gene name	Position	Number of nucleotides	Number of amino acids
1 (PapA)	1-684	684	-
2 (PapC) (inv)	949-1836	888	296
3 (PapB)	1873-2259	387	129
4 (PapM)	2259-2887	629	-

TABLE I

[077] Comparison of the product of frame 2 (TABLE I) with the protein sequences contained in the Genpro library shows a 27% homology with the region involved in the prephenate dehydrogenase activity of the bifunctional TyrA proteins of E. coli (Hudson and Davidson, 1984) and of Erwinia herbicola (EMBL data library, 1991). This region of TyrA catalyses aromatization of prephenate to form 4-hydroxyphenylpyruvate in the biosynthesis of tyrosine. A similar aromatization, proceeding from 4-deoxy-4-aminoprephenate and leading to 4-aminophenylpyruvate is very probably involved in the synthesis of DMPAPA. This reaction will be catalyzed by the product of frame 2, termed PapC (SEQ ID NO: 3).

[078] Comparison of the product of frame 3 (TABLE I) with the protein sequences contained in the Genpro library shows a 24 to 30% homology with the region involved in the chorismate mutase activity of the bifunctional TyrA and PheA proteins of E. coli (Hudson and Davidson, 1984) and of the TyrA protein of Erwinia herbicola. This region catalyzes isomerization of chorismate to form prephenate in the biosynthesis of tyrosine and

phenylalanine. A similar isomerization, proceeding from 4-deoxy-4-amino chorismate and leading to 4-deoxy-4-aminoprephenate, is very probably involved in the synthesis of DMPAPA. This reaction would be catalyzed by the product of frame 3, termed PapB (SEQ ID NO: 5).

[079] In the case of TyrA and PheA, the chorismate mutase and prephenate dehydratase, or prephenate dehydrogenase, activities are catalysed by the same protein. In S. pristinaespiralis, the chorismate mutase and prephenate dehydrogenase enzyme activities are catalysed by two separate proteins, i.e. PapB and PapC, respectively.

[080] The sequence homologies demonstrated for the PapB and PapC proteins demonstrate that these two proteins are involved, jointly with the PapA and PapM proteins, in the biosynthesis of the aromatic derivative DMPAPA. In the same way as for papA, disruption of the papB and papC genes should lead to the construction of S. pristinaespiralis strains which are incapable of producing PI but which are able, in the presence of novel precursors, to produce new PIs which are modified at the level of the DMPAPA residue.

[081] 1-2. Sequencing and analysis of the region upstream of the papA gene

[082] This region is contained between the snbA gene, which encodes 3-hydroxypicolinic acid AMP ligase and which is described in Patent PCT/FR93/00923, and the papA gene.

[083] The clonings were carried out as described in Example 1-1, proceeding from plasmid pVRC900 and cosmid pIBV2, which are described in Patent PCT/FR93/00923. The 1.3 kb XhoI-XhoI, 0.2 kb XhoI-XhoI, 3.3 kb XhoI-XhoI, 1.1 kb HindIII-PstI and 2.2 kb PstI-PstI fragments were subcloned into the M13mp18 and M13mp19 vectors. These different clonings made it possible to pass through all the cloning sites. The different inserts were sequenced as

described in 1-1 using, as synthesis primer, the universal primer or synthetic oligonucleotides which were complementary to a 20 nucleotide sequence in the insert to be sequenced.

[084] The overlap between these different inserts enabled the entire nucleotide sequence which is present between the snbA and papA genes (SEQ ID NO: 6) to be established.

[085] On the basis of this nucleotide sequence, it is possible to determine the open reading frames and to identify genes which are involved, in S. pristinaespiralis, in the biosynthesis of precursors of PI, as well as the polypeptides encoded by these genes.

[086] We have looked for the presence of open reading frames within the 4.5 kb XhoI-PstI fragment, which contains the nucleotide sequence between the snbA and papA genes, as described in Example 1.1. Using this method, it was possible to characterize four highly probable open reading frames within the 4.5 kb XhoI-PstI fragment, which frames are depicted in the table below (TABLE II). They are designated frames 1 to 4 in accordance with their position starting from the XhoI site. Their length in bases, and their position within the fragment (the XhoI site being situated at position I) has been indicated for each fragment; the number of amino acids within the encoded polypeptide has also been indicated for open reading frames 2 and 3. Frames 2, 3 and 4 are encoded by the same strand, and frame 1 is encoded by the complementary strand (Figure 5). Frames 1 and 4 correspond, respectively, to the N-terminal regions of the SnbA and PapA proteins, which were previously identified and described in patent PCT/FR93/00923.

Frame number and/or gene name	Position	Number of nucleotides	Number of amino acids
1 (SmbA) (inv)	1-329	329	-
2 (PipA)	607-1671	1065	355
3 (SnbF)	1800-2993	1194	398
4 (PapA)	3018-4496	1479	-

TABLE II

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[087] Comparison of the product of frame 2 (TABLE II) with the protein sequences contained in the Genpro library shows a 30% homology with ornithine cyclodeaminase of Agrobacterium tumefaciens (Schindler et al., 1989). This enzyme is involved in the final step in the catabolism of octopine; it converts L-ornithine into L-proline by means of cyclodeamination. Authors have demonstrated, by means of the incorporation of labelled lysine, that 4-oxopipecolic acid and 3-hydroxypicolinic acid, which are found both in PI_A and in virginiamycin S1, derived from lysine (Molinero et al., 1989; Reed et al., 1989). A reaction in which lysine was cyclodeaminated, similar to that described for ornithine, would lead to the formation of pipecolic acid. Taking this hypothesis into account, the product of frame 2 was termed PipA (SEQ ID NO: 8). The results of mutating the pipA gene, presented in 2-1, demonstrate that the pipA gene is involved solely in the synthesis of pipecolic acid, since this mutation has no effect on the biosynthesis of 3-hydroxypicolinic acid, which is also derived from lysine and of which pipecolic acid could have been a precursor.

[088] Comparison of the product of frame 3 (TABLE II) with the protein sequences contained in the Genpro library shows a 30 to 40% homology with several hydroxylases of the cytochrome P450 type, which hydroxylases are involved in the biosynthesis of secondary metabolites (Omer et al., 1990, Trower et al., 1992). Several hydroxylations can be envisaged in the biosynthesis of precursors of pristinamycin I, in particular in the biosynthesis of 3-HPA (hydroxylation of picolinic acid at the 3 position) and of 4-oxopipecolic acid (hydroxylation of pipecolic acid at the 4 position). The results of mutating the pipA gene, presented in 2-1-3, demonstrate that the product of frame 3 is involved in hydroxylation of the pipecolic acid residue of PI_E. The corresponding gene has therefore been termed snbF, and the corresponding protein SnbF (SEQ ID NO: 9 and SEQ ID NO: 10, respectively).

[089] 1-3. Sequencing the region downstream of the snbA gene.

[090] This region is included between the snbA gene, which encodes 3-hydroxypicolinic acid adenylate ligase, and the snbR gene, which encodes a membrane protein which is probably responsible for transport and for resistance to PI, with both genes having been described in Patent PCT/FR93/00923. Sequencing of this region was carried out using a fragment which was isolated from cosmid pIBV2, as described in Example 1-1.

[091] The 1.6 kb HindIII-BglII fragment was subcloned into the M13mp18 and M13mp19 vectors, proceeding from cosmid pIBV2. The insert was sequenced as described in 1-1, using, as synthesis primer, the universal primer or synthetic oligonucleotides which were complementary to a 20 nucleotide sequence of the insert to be sequenced. On the basis of the nucleotide sequence thus obtained (SEQ ID NO: 11), it is possible to determine the open reading frames and to identify, in S. pristinaespiralis, genes which are involved in the biosynthesis of the precursors of PI, as well as the polypeptides encoded by these genes. We looked for the

presence of open reading frames within the 1.6 kb HindIII-BglII fragment, which corresponds to the end of the snbA gene and its downstream region, as described in Example 1-1. A complete open coding frame, encoded by the same strand as the snbA gene (Figure 6), was detected. Relative to position 1, corresponding to the HindIII site, this frame starts at nucleotide 249, i.e. 30 nucleotides after the end of the snbA gene, and terminates at nucleotide 1481. It is 1233 nucleotides in size, corresponding to a protein of 411 amino acids.

[092] Comparison of the product of this open frame with the protein sequences contained in the Genpro library shows a 30 to 40% homology with a group of proteins which are probably involved (Thorson et al., 1993) in the transamination of intermediates in the biosynthesis of various antibiotics (DnrJ, EryC1, TylB, StrS and PrgL). Synthesis of the 3-HPA precursor, which appears to derive from lysine by a route other than cyclodeamination (see Examples 1-2 and 2-1), could necessitate a transamination step which can be catalyzed by the product of this frame 3, termed HpaA (SEQ ID NO: 13). The results of mutating this gene, presented in 2-2, demonstrate unequivocally that this gene is involved in synthesis of the 3-HPA precursor and confirm our hypothesis.

[093] The genes papB, papC, pipA, snbF and hpaA, which are described in the present invention, are grouped together with the snbA, papA and papM genes on a chromosomal region of approximately 10 kb (Figure 7). This confirms the presence of a cluster of genes which are involved in the biosynthesis of PI and its precursors. Studying regions upstream and downstream of this cluster should enable the other genes involved in the biosynthesis of PI precursors, in particular L-phenylglycine and L-2-aminobutyric acid, to be identified.

[094] **EXAMPLE 2: Construction of recombinant strains by means of disrupting identified genes.**

[095] This example illustrates how it is possible to demonstrate involvement of the genes described in Example 1 in the biosynthesis of pristinamycin precursors, and also to construct S. pristinaespiralis strains which are able to produce novel pristinamycins. These strains are obtained by disrupting the genes which are involved in the biosynthesis of the residue which it is desired to replace, and the novel pristinamycins are produced by supplementing these mutants with novel precursors.

[096] Strain SP92::pVRCC508, which is employed in the present invention to produce novel derivatives of PI by replacing the precursor DMPAPA with other molecules, is described in Patent PCT/FR93/0923. It is obtained by disrupting, by means of simple crossing over, the papA gene, which is involved in the biosynthesis of the precursor of DMPAPA and is thought to participate in an early step relating to the transamination of chorismate. This disruption has a polar character since, in this mutant, expression of the papM gene (PCT/FR93/0923), which is situated 1.5 kb downstream of the papA gene and is involved in the double methylation of 4-amino-L-phenylalanine to form DMPAPA, is very reduced. Thus, assaying the activity of the SAM-dependant methylation enzyme for converting 4-amino-L-phenylalanine (PAPA) into DMPAPA indicates that mutant SP92::pVRC508 has an activity which is less than 5% of the activity of the wild-type strain.

[097] In the present invention, this strain, SP92::pVRC508, can be used, under appropriate fermentation conditions and supplementation conditions, to produce novel pristinamycins which are modified at the level of the DMPAPA residue, as will be explained in

Example 3. Mutants having the same phenotype can be obtained by disrupting the papB or papC genes described in the present invention.

[098] Another type of S. pristinaespiralis strain, whose papA gene is disrupted and which possesses the same phenotype as strain SP92::pVRC508, was obtained in a similar manner by disrupting the papA gene by means of double crossing over. This construction was carried out starting with a 4.6 kb SphI-HindIII fragment, which fragment was isolated from cosmid pIBV2 and contains the 3' region of the pipA gene, the entire snbF and papA genes and the 3' part of the papC gene. This fragment was cloned into the suicide vector pDH5, which vector is only able to replicate in E. coli but carries a resistance marker which is expressed in Streptomyces (the gene for resistance to thiostrepton or to nohiheptide, tsr). This vector, pDH5, was developed by Wohlebben et al (1991 Nucleic Acid Res. 19, 727-731). A BclI-BclI deletion of 1.1 kb was then made in the papA gene, and a 2.2 kb HindIII-HindIII fragment, carrying the amR gene (resistance to geneticin and to apramycin), was introduced after the cohesive ends had been filled in. The recombinant vector was termed pVRC414 and is depicted in Figure 12. After transforming the pristinamycin-producing strain with plasmid pVRC414, transformants which were resistant to geneticin and sensitive to thiostrepton were isolated and analysed. These clones are the result of a double homologous recombination between the S. pristinaespiralis DNA regions of plasmid pVRC414 and the corresponding chromosomal region of S. pristinaespiralis, as described in Figure 13. One of these clones was termed SP212. Its phenotype is identical to that of strain SP92::pVRC508 as regards the absence of any production of PI and the ability of the strain to produce new antibiotics in the presence of novel precursors. Advantageously, this type of strain, which is obtained by double crossing over, is more stable than the strains which are obtained by simple crossing over.

[099] 2-1. Construction of a mutant of S. pristinaespiralis SP92 whose pipA gene is disrupted.

[0100] This example illustrates how it is possible, by means of disrupting the pipA gene, to construct a strain of S. pristinaespiralis SP92 which no longer produces PI under standard fermentation conditions and which is able to produce new pristinamycins, which are modified at the level of the 4-oxopipecolic acid residue of PIA, when novel precursors are added to the fermentation.

[0101] It was constructed using a suicide vector, the vector pUC1318, which only replicates in E. coli. This vector does not carry any resistance marker which is expressed in Streptomyces. Its presence in the genome of Streptomyces can only be detected by colony hybridization.

[0102] 2-1-1. Construction of plasmid pVRC420:

[0103] This example illustrates how it is possible to construct a plasmid which does not replicate in S. pristinaespiralis SP92 and which can be employed to disrupt the pipA gene by means of double homologous recombination.

[0104] Plasmid pVRC420 was constructed in order to produce the chromosomal mutant of SP92 in which the pipA gene is disrupted, proceeding from cosmid pIBV2, which is described in Patent PCT/FR93/0923. Cosmid pIBV2 was cut with the restriction enzyme PstI and, after the fragments, thus generated, had been separated by electrophoresis on a 0.8% agarose gel, a 2.8 kb PstI-PstI fragment, containing the start of the snbA and snbF genes and the whole of the pipA gene, was isolated and purified using Geneclean (Bio101, La Jolla, California). 50 ng of vector pUC1318, which had been linearized by digesting with PstI, were ligated to 200 ng of the 2.8 kb fragment, as described in Example 1. A clone carrying the desired fragment was isolated

following transformation of the strain TG1 and selection on LB + 150 μg/ml ampicillin + X-gal + IPTG medium. The recombinant plasmid was termed pVRC415 (Figure 8). A cassette containing the am^R gene, encoding resistance to apramycin or to geneticin (Kuhstoss et al., 1991), was then introduced into the unique HindIII site of plasmid pVRC415, this site being situated 530 bp downstream of the start of the pipA gene. This construction was effected as follows. A 2.5 kb DNA fragment, containing the am^R gene, the PermE promoter (Bibb et al., 1985) and the first 158 amino acids of the gene for resistance to erythromycin , ermE, was isolated by means of a SalI-BglII double digestion of a plasmid which was derived from plasmids pIJ4026 (plasmid carrying the ermE gene under the control of the PermE promoter) and pHP45Ωam^R. After filling in the SalI and BglII protruding 5' cohesive ends using Klenow enzyme in accordance with the protocol described by Maniatis et al., 1989, the fragment containing the am^R gene was cloned into the HindIII site of plasmid pVRC415, whose protruding 5' cohesive ends had also been filled in with Klenow enzyme as previously described. The recombinant plasmid thus obtained was designated pVRC420. Its restriction map is depicted in Figure 9.

[0105] 2-1-2. Isolation of mutant SP92pipA::Ωam^R, whose pipA gene is disrupted by homologous recombination.

[0106] This example illustrates how the mutant of S. pristinaespiralis SP92 whose pipA gene is disrupted was constructed.

[0107] This mutant was isolated by transforming strain SP92 with the suicide plasmid pVRC420.

[0108] The preparation of protoplasts, their transformation and extraction of the total DNA from the recombinant strains were all effected as described by Hopwood et al. (1985).

[0109] The strain SP92 was cultured, at 30°C for 40 hours, in YEME medium (Hopwood et al., 1985), 34% sucrose, 5 mM $MgCl_2$ and 0.25% glycine. The mycelium was protoplasted in the presence of lysozyme, and 5 × 1 μg of pVRC420 were used to transform (by the method employing PEG) the protoplasts. After one night in which the protoplasts were regenerated on R2YE medium (D. Hopwood et al. 1985), the recombinants were selected by spreading on 3 ml of SNA medium (D. Hopwood et al. 1985) containing 1,500 μg/ml geneticin.

[0110] 100 clones which were resistant to geneticin were isolated from the 5 transformations that were carried out. These recombinants arise from integration, by means of simple or double homologous recombination between the pipA gene which is carried by the chromosome of strain SP92 and the parts of the pipA gene which are contained in the 5.3 kb fragment carried by the suicide plasmid pVRC420. In order to select the recombinants which were obtained by double crossing over (that is which did not contain the pUC1318 part of plasmid pVRC420 in their genome), colony hybridizations were carried out on 90 clones using pUC19 labelled with [α-^{32}P]dCTP as the probe, as described in Maniatis et al (1989). 10 clones were selected which were resistant to geneticin but which did not hybridize the vector pUC19. The spores of the recombinants were isolated by streaking and growing on HT7 medium containing 10 μg/ml geneticin, and restreaked on the same medium in order to obtain isolated colonies. In order to verify the position at which plasmid pVRC420 was integrated, various Southerns of the total DNA from several recombinant clones, purified as described by Hopwood et al. 1985, were carried out, with hybridization to the 2.8 kb PstI-PstI fragment, which was used as a probe after having been labelled with [α-^{32}P]dCTP. The results confirm that these recombinants were obtained by double crossing over between vector pVRC420 and the chromosome of strain SP92, resulting in replacement of the 2.8 kb PstI-PstI fragment, containing

the pipA gene, by a 5.3 kb PstI-PstI fragment containing the pipA gene which is disrupted by introduction of the am^R gene. One of these mutants was designated SP92pipA::Ωam^R.

[0111] 2-1-3. Production of pristinamycins using mutant SP92pipA::Ωam^R.

[0112] This example illustrates how it is established that the mutant of S. pristinaespiralis SP92 whose pipA gene is disrupted by integration of plasmid pVR420 on the one hand no longer produces PI under standard fermentation conditions and on the other hand exhibits a high level of production of a minor form of the B components of streptogramins in which 4-oxopipecolic acid is replaced by pipecolic acid.

[0113] Mutant SP92pipA::Ωam^R, as well as strain SP92 in the role of a control strain, were cultrured in liquid production medium. The fermentation was carried out as follows: 0.5 ml of a suspension of spores from the abovementioned strain are added, under sterile conditions, to 40 ml of inoculum medium in a 300 ml baffled Erlenmeyer flask. The inoculum medium is made up of g/l corn steep, 15 g/l sucrose, 10 g/l $(NH_4)_2SO_4$, 1 g/l K_2HPO_4, 3 g/l NaCl, 0.2 g/l $MgSO_4$-$7H_2O$ and 1.25 g/l $CaCO_3$. The pH is adjusted to 6.9 using sodium hydroxide solution before introducing the calcium carbonate. The Erlenmeyer flasks are shaken at 27°C for 44 h on a rotating shaker at a speed of 325 rpm. 2.5 ml of the previous culture, which is 44 hr old, are added under sterile conditions to 30 ml of production medium in a 300 ml Erlenmeyer flask. The production medium is made up of 25 g/l soya flour, 7.5 g/l starch, 22.5 g/l glucose, 3.5 g/l fodder yeast, 0.5 g/l zinc sulphate and 6 g/l calcium carbonate. The pH is adjusted to 6.0 with hydrochloric acid before introducing the calcium carbonate. The Erlenmeyer flasks are shaken for 24, 28 and 32 hours at 27°C. At each time point, 10 g of must are weighed into a smooth Erlenmeyer flask to which 20 ml of mobile phase, consisting of 34% of acetonitrile and 66% of a solution of 0.1 M KH_2PO_4 (adjusted to pH 2.9 with concentrated H_3PO_4) are added for extracting

the pristinamycins. After shaking, the whole is centrifuged and the pristinamycins contained in the supernatant are assayed by HPLC by means of injecting 150 µl of the centrifugation supernatant onto a Nucleosil 5-C8 column of 4.6 x 150 mm, which is eluted with a mixture of 40% acetonitrile and 60% 0.1 M phosphate buffer, pH 2.9. The I pristinamycins are detected by means of their UV absorbance at 206 nm.

[0114] The results demonstrated that, under the fermentation conditions employed, mutant SP92pipA::Ωam^R did not produce PI at 24, 28 or 32 hrs of fermentation, while control strain SP92 produced a quantity of PI which was standard for the 3 times which were tested. The quantity of PII which was produced remained the same for the two strains. Mutant SP92pipA::Ωam^R is definitely blocked at a step in the biosynthesis of PI. Fermentation complementation tests were carried out by adding different precursors of PI, separately or together, to the culture in production medium after 16 hours. The results of these complementations demonstrated that when 100 mg/l pipecolic acid and 100 mg/l DMPAPA are added simultaneously to the fermentation medium, the mutant produces what is normally a minor derivative of PI, i.e. PI_E (which is produced by SP92 in a quantity which is less than 5%) at a level which is equivalent to the production of PI_A by the control strain. This production does not take place if the pipecolic acid and the DMPAPA are added separately. PI_E differs from PI_A (major component of PI) in the absence of the keto function in the 4 position on the pipecolic acid. The fact that mutant SP92pipA::Ωam^R can only be complemented by adding pipecolic acid and DMPAPA simultaneously indicates that the papA, and probably the papB and papM genes were disrupted by a polar effect of the construct. Thus, all these genes are situated downstream of pipA and are probably cotranscripts together with pipA. Disruption of the latter therefore leads to disruption of the pap genes and, consequently, absence of DMPAPA synthesis. The fact

that complementation of mutant SP92pipA::Ωam^R with pipecolic acid results in the production of PI_E and not PI_A leads to two conclusions: the first is that construction of the PI cycle is achieved by incorporating pipecolic acid and not 4-oxopipecolic acid and that a hydroxylation generating the keto function in the 4 position then takes place subsequently. The second is that this hydroxylation is probably carried out by the enzyme SnbF whose structural gene is situated directly downstream of the pipA gene. Thus, the obvious polarity of the disruption of the pipA gene on the pap genes probably involves a polar effect on the snbF gene, which is situated between pipA and the pap genes, which is manifested in inhibition of the function of hydroxylation of the pipecolic acid residue of PI_E to form 4-hydroxypipecolic acid, which is found in PI_F and PI_G (Figure 2) and then oxidized to 4-oxopipecolic acid in PI_A.

[0115] Preparing a mutant of this nature made it possible to construct a strain of S. pristinaespiralis which is unable to produce PI except in the presence of the PI precursors DMPAPA and pipecolic acid, using which it is able to produce, in a quantity equivalent to that of the starting strain, what is normally a minor derivative of PI within the pristinamycin mixture. Similarly, in the presence of novel precursors, or of a mixture of novel precursors and of precursors which are normally present in PI, this strain will be able to produce new pristinamycins which are modified in either DMPAPA or 4-oxopipecolic acid or in both these residues.

[0116] 2-2. Construction of a mutant of S. pristinaespiralis SP92 whose hpaA gene is disrupted.

[0117] This example illustrates how it is possible, by means of disrupting the hpaA gene, to construct a strain of S. pristinaespiralis SP92 which no longer produces PI under standard

fermentation conditions and which is able to produce new pristinamycins, which are modified at the level of the 3-HPA precursor, when novel precursors are added to the fermentation.

[0118] This mutant was constructed using a plasmid which does not replicate in S. pristinaespiralis SP92 and which can be used for disrupting the hpaA gene by means of double homologous recombination.

[0119] 2-2-1. Construction of the suicide plasmid pVRC421

[0120] Plasmid pVRC421 was constructed using a suicide vector which, while only being able to replicate in E. coli, carries a resistance marker which is expressed in Streptomyces, i.e. the gene for resistance to thiostrepton or to nosiheptide, tsr. This vector, pDH5, was developed by Hillemann et al. (1991).

[0121] Plasmid pVRC421 was constructed in order to produce the chromosomal mutant of SP92 whose hpaA gene is disrupted, making use of cosmid pIBV2, which is described in Patent PCT/FR93/0923. pIBV2 was digested with the restriction enzyme SphI and, after having separated the fragments, thus generated, by means of electrophoresis on a 0.6% agarose gel, a 4.8 kb SphI-SphI fragment, containing the whole of the hpaA gene and virtually the whole of the snbA gene, was isolated and purified using Geneclean as described above. 50 ng of the vector pDH5, linearized by digesting with SphI, were ligated to 200 ng of the 4.8 kb fragment, as subsequently described. A clone harbouring the desired fragment was isolated after transforming the strain TG1 and selecting on LB + 150 μg/ml ampicillin + IPTG + X-gal medium. The recombinant plasmid was designated pVRC411 (Figure 10). A cassette containing the gene am^R, encoding resistance to apramycin or to geneticin, was then introduced into the unique PflmI site of plasmid pVRC411, this site being situated 610 bp downstream of the start of the hpaA gene. This construct was produced as follows. A 2.2 kb DNA fragment, containing the am^R gene, was

isolated following digestion of the plasmid pHP45Ωam^R, containing the am^R gene, with HindIII. After filling in the HindIII protruding 5' cohesive ends using Klenow enzyme according to the protocol described by Maniatis et al. 1989, the fragment containing the am^R gene was cloned into the PflmI site of plasmid pVRC411, whose protruding 3' cohesive ends had been rendered blunt using the enzyme T4 polymerase as described in Maniatis et al. 1989. The recombinant plasmid thus obtained was termed pVRC421. Its restriction map is depicted in Figure 11.

[0122] 2-2-2. Isolation of mutant SP92hpaA::Ωam^R, whose hpaA gene is disrupted by means of homologous recombination.

[0123] This example illustrates how the mutant of S. pristinaespiralis SP92 whose hpaA gene is disrupted was constructed.

[0124] This mutant was isolated by transforming strain SP92 with the suicide plasmid pVRC421.

[0125] The protoplasts were prepared and transformed as described previously.

[0126] Strain SP92 was cultured, at 30°C for 40 hours, in YEME medium, 34% sucrose, 5 mM $MgCl_2$, 0.25% glycine. The mycelium was protoplasted in the presence of lysozyme, and 5 x 1 μg of pVRC421 were employed for transforming (by the method using PEG) the protoplasts. After one night for regenerating the protoplasts on R2YE medium, the recombinants were selected by spreading on 3 ml of SNA medium containing 1,500 μg/ml geneticin.

[0127] 600 clones which were resistant to geneticin were isolated from the 5 transformations which were carried out. These recombinants result from integration by means of simple or double homologous recombination between the hpaA gene carried by the chromosome of strain SP92 and the 6 kb fragment of the suicide plasmid pVRC421. In order to select the recombinants obtained by double crossing over (that is, the clones which no longer contain, in

their genome, the pDH5 moiety of plasmid pVRC421), the clones were subcultured on HT7 medium containing 400 μg/ml thiostrepton. 6 clones which were resistant to geneticin but sensitive to thiostrepton were selected. The spores of the recombinants were selected by streaking and growth on HT7 medium containing 10 μg/ml geneticin, and restreaked on the same medium in order to obtain isolated colonies. In order to verify the position of integration of plasmid pVRC421, various Southerns of the total DNA from the 6 recombinant clones, purified as described by Hopwood et al. 1985, were carried out with hybridization to the 4.8 kb SphI-SphI fragment, which was used as the probe after having been labelled with [α-^{32}P]dCTP. The results confirm that these recombinants were obtained by double crossing over between the vector pVRC421 and the chromosome of the SP92 strain, resulting in replacement of the 4.8 kb SphI-SphI fragment, containing the hpaA gene, by a 6 kb SphI-SphI fragment which contains the hpaA gene disrupted by the am^R gene. One of these mutants was designated SP92hpaA::Ωam^R.

[0128] 2-2-3. Production of pristinamycins by mutant SP92hpaA::Ωam^R.

[0129] This example illustrates how it is established that the mutant of S. pristinaespiralis SP92 whose hpaA gene is disrupted by integration of plasmid pVR421 no longer produces PI under the standard fermentation conditions.

[0130] Mutant SP92hpaA::Ωam^R, and also strain SP92 in the role of control strain, were cultured in liquid production medium. The fermentation was carried out as described in Example 2-1-3, and the pristinamycins were then extracted and assayed as previously described. The results demonstrated that, under the fermentation conditions employed, mutant SP92hpaA::Ωam^R did not produce PI, either at 24, 28 or 32 hrs of fermentation, whereas the control strain produced a quantity of PI which was standard for the 3 time points tested. The quantity of PII produced remained the same for the two strains. Mutant SP92hpaA::Ωam^R is

definitely blocked at a step in the biosynthesis of PI. Complementary fermentation tests were carried out by adding different precursors of PI, separately or together, to the culture in production medium after 16 hours. When 100 mg/l 3-hydroxypicolinic acid are added to the fermentation medium, the mutant then produces PI_A at a level which is equivalent to the production of PI by the control strain. The fact that mutant SP92hpaA::Ωam^R can only be complemented by adding 3-hydroxypicolinic acid demonstrates that the hpaA gene is involved in the synthesis of this precursor.

[0131] Construction of this mutant made it possible to produce a strain of S. pristinaespiralis which is mutated as regards its production of PI but which, in the presence of the precursor 3-HPA, is capable of producing PI in a quantity equivalent to that produced by the starting strain. In the same way as in the preceding examples, it can be envisaged that it should be possible, using a mutant of this nature in the presence of novel precursors, to produce new pristinamycins which are modified at the level of the 3-hydroxypicolinic acid residue.

[0132] **EXAMPLE 3: Production of compounds of the general formula I by the mutant SP92::pVRC508.**

[0133] This example illustrates how the mutant of S. pristinaespiralis SP92 whose papA gene is disrupted by integration of plasmid pVRC508 is able to synthesize new streptogramins in the presence of precursors which are added to the production medium. These precursors can be derivatives of amino acids and, more particularly, of phenylalanine, but also of α-ketocarboxylic acids and, more particularly, of phenylpyruvic acid.

[0134] The mutant SP92::pVRC508 was cultured in liquid production medium. The fermentation was carried out as follows: 0.5 ml of a suspension of spores from the previously mentioned strain is added, under sterile conditions, to 40 ml of inoculum medium in a 300 ml

baffled Erlenmeyer flask. The inoculum medium is made up of 10 g/l corn steep, 15 g/l sucrose, 10 g/l $(NH_4)_2SO_4$, 1 g/l K_2HPO_4, 3 g/l NaCl, 0.2 g/l $MgSO_4$-$7H_2O$ and 1.25 g/l $CaCO_3$. The pH is adjusted to 6.9 with sodium hydroxide solution before introducing the calcium carbonate. The Erlenmeyer flasks are shaken at 27°C for 44 h on a rotating shaker at a speed of 325 rpm. 2.5 ml of the previous culture, which is 44 h old, are added, under sterile conditions, to 30 ml of production medium in a 300 ml Erlenmeyer flask. The production medium consists of 25 g/l soya flour, 7.5 g/l starch, 22.5 g/l glucose, 3.5 g/l fodder yeast, 0.5 g/l zinc sulphate and 6 g/l calcium carbonate. The pH is adjusted to 6.0 with hydrochloric acid before introducing the calcium carbonate. The Erlenmeyer flasks are shaken at 27°C on a rotating shaker at a speed of 325 rpm. After 16 h, 1 ml of a solution of one of the precursors listed in Table 3 (generally 5 or 10 g/l) is added to the culture. The latter is terminated 8 or 24 h later. The volume of the must is measured immediately, and 2 volumes of mobile phase, consisting of 34% acetonitrile and 66% of a solution of 0.1 M KH_2PO_4 (adjusted to pH 2.9 with concentrated H_3PO_4) are added to it for extracting the pristinamycins. After shaking, the whole is centrifuged and the pristinamycins contained in the supernatant are extracted and purified as described in Example 4. They are also assayed by HPLC by means of injecting 150 μl of the centrifugation supernatant onto a Nucleosil 5-C8 4.6 x 150 mm column, which is eluted with a mixture of 40% acetonitrile and 60% 0.1 M phosphate buffer, pH 2.9. The new I pristinamycins are detected by means of their UV absorbance at 206 nm and, where appropriate, by means of their fluorescence emission (370 nm filter, excitation at 306 nm).

PRECURSOR	ORIGIN
phenylalanine	Janssen
4-dimethylaminophenylalanine	Example 33
4-methylaminophenylalanine	Example 34-1
4-aminophenylalanine	Janssen 22.794.96
4-diethylaminophenylalanine	Example 33
4-ethylaminophenylalanine	Example 33
4-methylthiophenylalanine	Example 33
4-methylphenylalanine	J.P.S101-312-4/ Example 33
4-methoxyphenylalanine	Janssen 16.975.97
4-trifluoromethoxyphenylalanine	Example 34-8
4-methoxycarbonylphenylalanine	Example 33
4-chlorophenylalanine	Janssen 15.728.14
4-bromophenylalanine	Janssen 22.779.81
4-iodophenylalanine	Bachem F 1675
4-trifluoromethylphenylalanine	P.C.R. Inc. 12

	445-3
4-tert-butylphenylalanine	Example 35-1
4-isopropylphenylalanine	Example 36-1
3-methylaminophenylalanine	Example 35-3
3-methoxyphenylalanine	J.P.S. 101-313-2
3-methylthiophenylalanine	Example 34-11
3-fluoro-4-methylphenylalanine	Example 34-5
4-tert-butylphenylpyruvic acid	Example 33
4-methylaminophenylpyruvic acid	Example 34-4
2-napthylphenylalanine	Bachem F 1865
4-fluorophenylalanine	Bachem F 1535
PRECURSOR	ORIGIN
3-fluorophenylalanine	Bachem F 2135
3-ethoxyphenylalanine	Example 37-1
2,4-dimethylphenylalanine	Example 33
3,4-dimethylphenylalanine	Example 33
3-methylphenylalanine	Example 33
4-phenylphenylalanine	Example 33
4-butylphenylalanine	Example 36-3
2-thienyl-3-alanine	Aldrich 28.728.8
3-trifluoromethylphenylalanine	Example 33
3-hydroxyphenylalanine	Aldrich T

	9.039.5
3-ethylaminophenylalanine	Example 35-6
4-aminomethylphenylalanine	Example 33
4-allylaminophenylalanine	Example 38-2
4-diallylaminophenylalanine	Example 38-1
4-allylethylaminophenylalanine	Example 39-4
4-ethylpropylaminophenylalanine	Example 39-6
4-ethylisopropylaminophenylalanine	Example 39-1
4-ethylmethylcyclopropylamino-phenylalanine	Example 39-8
4-(1pyrrolidinyl)phenylalanine	Example 40-1
4-O-allyltyrosine	Example 33
4-O-ethyltyrosine	Example 33
4-ethylthiophenylalanine	Example 33
4-ethylthiomethylphenylalanine	Example 41-1
4-O-(2-chloroethyl)tyrosine	Example 42-1
4-acetylphenylalanine	Example 33
4-ethylphenylalanine	Example 33
3-dimethylaminophenylalanine	Example 35-10

TABLE III

[0135] The following table (TABLE IV) indicates the relative retention times of the new PI's which are produced, taking PI_A as the reference. The absolute retention times were

determined at 25°C in the HPLC system described above; they vary slightly from one injection to another and also in accordance with temperature.

Precursor	t_R (relative retention time) of the new PI (Neo PI)		
	Neo PI_A	Neo PI_H	Other neo PI
4-methylaminophenylalanine	0.85		
4-aminophenylalanine	0.64		
4-methylthiophenylalanine	1.93	2.73	1.63
4-methylphenylalanine	1.77	2.65	
4-methoxyphenylalanine	1.46		
4-methoxycarbonylphenylalanine	1.49		
4-chlorophenylalanine	2.04		
4-bromophenylalanine	2.16		
4-iodophenylalanine	2.42		
4-trifluoromethylphenylalanine	2.56	3.74	
4-tert-butylphenylalanine	3.34		
4-isopropylphenylalanine	2.80		4.35
3-methylaminophenylalanine	1.15		
3-methoxyphenylalanine	1.49	2.04	
3-fluoro-4-methylphenylalanine	2.93		
4-tert-butylphenylpyruvic acid	3.34		

4-methylaminophenylpyruvic acid	0.85		
4-ethylaminophenylalanine	0.94		
4-diethylaminophenylalanine	0.61		
4-allylaminophenylalanine	1.83		
4-diallylaminophenylalanine	2.64		
4-allylethylaminophenylalanine	2.4		
4-ethylpropylaminophenylalanine	1.06		
4-ethylisopropylaminophenylalanine	0.89		
4-ethylmethylcyclopropylaminophenylalanine	1.1		
4-(1-pyrrolidinyl)phenylalanine	2.0		
4-O-trifluoromethyltyrosine	2.42		
4-O-allyltyrosine	2.62		
4-O-ethyltyrosine	2.2		
4-ethylthiophenylalanine	1.96		
4-methylthiomethylphenylalanine	1.98		
4-O-(2-chloroethyl)tyrosine	2.45		
4-acetylphenylalanine	1.61		
4-ethylphenylalanine	1.86	2.40	
3-dimethylaminophenylalanine	1.49		
3-methylthiophenylalanine	1.93		
3-O-ethyltyrosine	1.78		

TABLE IV

[0136] The new PI, with a t_R of 4.35, for 4-isopropylphenylalanine corresponds to a neo PI_E which is described in Example 14.

[0137] The new PI, with a t_R of 1.63, for 4-methylthiophenylalanine corresponds to a 5γ-hydroxy neo PI_H, which is described in Example 5.

[0138] The mutant SP92::pVRC508 was otherwise fermented in the presence of 4-dimethylaminophenylalanine. Under these conditions of complementation, mutant SP92::pVRC508 produces a quantity of I_A pristinamycins which is equivalent to that produced by strain SP92.

[0139] EXAMPLE 4: Preparation of pristinamycin I_B [4ζ-methylamino- de(4ζ-dimethylamino)pristinamycin I_A] and of 4ζ-amino-de(4ζ-dimethylamino)pristinamycin I_A

[0140] 4.1: Preparation of pristinamycin I_B [4ζ-methylamino-de(4ζ-dimethylamino)pristinamycin I_A]

[0141] The strain SP92::pVRC508 is cultured in production medium, using 60 Erlenmeyer flasks as described in Example 3, with 1 ml of a 10 g/l aqueous solution of (R,S)-4-methylaminophenylalanine, synthesized as in Example 34-1, being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichlormethane and is successively eluted with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing pristinamycin I_B are combined and evaporated. The dry residue is taken up in 6 ml of a mixture

of 65% water and 35% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture of 65% 100 mM phosphate buffer, pH 2.9, and 35% acetonitrile. The fractions containing pristinamycin I_B are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried on sodium sulphate and then evaporated. 52 mg of pristinamycin I_B are obtained.

[0142] NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.71 (dd, J=16 and 6 Hz, 1H, 5 β_2), 0.92 (t, J=7.5 Hz, 3H: CH_3 2 γ), from 1.10 to 1.40 (mt, 2H: 3 β_2 and 3 γ_2), 1.34 (d, J=7.5 Hz, 3H: CH_3 1 γ), from 1.50 to 1.85 (mt, 3H: 3 γ_1 and CH_2 2 β), 2.03 (mt, 1H, 3 β_1), 2.22 (mt, 1H, 5 δ_2), 2.33 (broad d, J=16 Hz, 1H: 5 δ_1), 2.40 (d, J=16 Hz, 1H: 5 β_1), 2.82 (mt, 1H: 5 ε_2), 2.81 (s, 3H: 4 NCH_3 in the para position of the phenyl), 2.90 (dd, J=12 and 4 Hz, 1H: 4 β_2), 3.29 (s, 3H: 4 NCH_3) from 3.20 to 3.45 and 3.60 (2 mts, 1H each: CH_2 3 δ), 3.40 (t, J=12 Hz, 1H: 4 β_1), 4.57 (dd, J=7 and 8 Hz, 1H, 3α), 4.75 (broad dd, J=13 and 7 Hz, 1H: 5 ε_1), 4.83 (mt, 1H: 2α), 4.89 (broad d, J=10 Hz, 1H: 1α), 5.24 (dd, J=12 and 4 Hz, 1H: 4 α), 5.32 (broad d, J=6 Hz, 1H: 5 α), 5.89 (d, J=9 Hz, 1H: 6 α), 5.90 (broad q, J = 7.5 Hz, 1H: 1 β), 6.53 (d, J=9 Hz, 1H: NH 2), 6.53 (d, J=8 Hz, 2H: 4 ε), 7.03 (d, J=8 Hz, 2H: 4 δ), from 7.10 to 7.35 (mt, 5H: aromatic H 6), 7.46 (mt, 2H: $1'H_5$ and $1'H_4$), 7.85 (dd, J=5.5 and 2 Hz, 1H: $1'H_6$), 8.44 (d, J=10 Hz, 1H: NH 1), 8.76 (d, J=9 Hz, 1H: NH 6), 11.63 (s, 1H: OH).

[0143] 4.2: Preparation of 4ζ-amino-de(4ζ-dimethylamino)pristinamycin I_A

[0144] Strain SP92::pVRC508 is cultured in production medium, using 60 Erlenmeyer flasks as described in Example 3, with 1 ml of a 5 g/l aqueous solution of (S)-4-aminophenylalanine being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9 and 34% acetonitrile, and then centrifuged. The

supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. The dry residue is taken up in 6 ml of a mixture consisting of 65% water and 35% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 65% 100 mM phosphate buffer, pH 2.9, and 35% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 5 mg of 4ζ-amino-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

[0145] NMR spectrum: ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.72 (dd, J=16 and 5.5 Hz, 1H, 5 β_2), 0.90 (t, J=7.5 Hz, 3H: CH_3 2 γ), from 1.10 to 1.40 (mt, 2H: 3 β_2 and 3 γ_2), 1.33 (d, J=7.5 Hz, 3H: CH_3 1 γ), from 1.50 to 1.85 (mt, 3H: 3 γ_1 and CH_2 2 β), 2.02 (mt, 1H, 3 β_1), 2.19 (mt, 1H, 5 δ_2), 2.33 (broad d, J=16 Hz, 1H: 5 δ_1), 2.42 (d, J=16 Hz, 1H: 5 β_1), 2.81 (dt, J=13 and 4 Hz, 1H: 5 ε_2), 2.90 (dd, J=12 and 4 Hz, 1H: 4 β_2), 3.24 (s, 3H: NCH_3 4), from 3.20 to 3.40 and 3.54 (2 mts, 1H each: CH_2 3 δ), 3.30 (t, J=12 Hz, 1H: 4 β_1), 3.72 (unres.comp., 2H: $ArNH_2$), 4.54 (dd, J=7.5 and 7 Hz, 1H, 3 α), 4.73 (broad dd, J=13 and 8 Hz, 1H: 5 ε_1), 4.82 (mt, 1H: 2α), 4.89 (broad d, J=10 Hz, 1H: 1α), 5.22 (dd, J=12 and 4 Hz, 1H: 4 α), 5.32 (broad d, J=5.5 Hz, 1H: 5 α), 5.89 (mt, 2H: 6 α and l β), 6.51 (d, J=9.5 Hz, 1H: NH 2) 6.61 (d J=8 Hz, 2H: 4 ε), 6.98 (d, J=8 Hz, 2H: 4δ), from 7.15 to 7.35 (mt, 5H: aromatic H 6), 7.45 (dd, J=8.5 and 1.5 Hz, 1H: l'H_4),

7.48 (dd, J=8.5 and 4 Hz, 1H: 1'H_5), 7.82 (dd, J=4 and 1.5 Hz, 1H: 1'H_6), 8.43 (d, J=10 Hz, 1H: NH 1), 8.76 (d, J=9.5 Hz, 1H: NH 6), 11.63 (s, 1H: OH).

[0146] **Example 5: Preparation of 4ζ-methylthio-de(4ζ-dimethylamino)pristinamycin I_A, of 4ζ-methylthio-de(4ζ-dimethylamino)pristinamycin I_H, and of 5-γ-hydroxy-4ζ- methylthio-de(4ζ-dimethylamino)pristinamycin I_H**

[0147] Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution of (R,S)-4-methylthiophenylalanine, synthesized as in Example 33, in 0.1N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. 65 mg of dry residue are obtained. This is taken up in 6 ml of a mixture consisting of 60% water and 40% acetonitrile and injected in two batches onto a semi-preparative Nucleosil 7 μ C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 45 mg of 4ζ-methylthio-de (4ζ-dimethylamino)pristinamycin I_A are obtained.

[0148] NMR spectrum: ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.68 (dd, J=16 and 5.5 Hz, 1H 5 β_2), 0.93 (t, J=7.5 Hz, 3H: CH_3, 2 γ), 1.13 (mt, 1H: 3 β_2), from 1.25 to 1.40 (mt, 1H: 3 γ_2), 1.33 (d, J=7.5 Hz, 3H: CH_3 1 γ), from 1.55 to 1.85 (mt, 3H: 3 γ_1, and CH_2 2 β), 2.02 (mt, 1H, 3 β_1), 2.18 (mt, 1H, 5 δ_2), 2.38 (broad d, J=16.5 Hz, 1H: 5 δ_1), 2.46 (s, 3H: SCH_3), 2.48 (d, J=16 Hz, 1H, 5 β_1), 2.85 (dt, J=13.5 and 4 Hz, 1H: 5 ε_2), 3.00 (dd, J=12 and 5 Hz, 1H: 4 β_2), 3.23 (s, 3H: NCH_3, 4), 3.37 (t, J=12 Hz, 1H: 4 β_1), 3.37 and 3.58 (2 mts, 1H each: CH_2 3 δ), 4.55 (t, J=7.5 Hz, 1H, 3 α), 4.77 (broad dd, J=13.5 and 8 Hz, 1H: 5 ε_1), 4.86 (mt, 1H: 2α), 4.89 (dd, J=10 and 1.5 Hz, 1H: l α), 5.30 (broad d, J=5.5 Hz, 1H: 5 α), 5.32 (dd, J=12 and 5 Hz, 1H: 4 α), 5.90 (d, J=9.5 Hz, 1H: 6 α), 5.92 (dq, J=7.5 and 1.5 Hz, 1H: 1 β), 6.55 (d, J=9.5 Hz, 1H: NH 2), 7.13 (d, J=8 Hz, 2H: 4 δ), from 7.15 to 7.35 (mt, 5H: aromatic H 6), 7.19 (d, J=8 Hz, 2H: 4 ε), 7.45 (mt, 2H: 1'H_4 and H_5), 7.76 (t, J=5 Hz, l'H_6), 8.42 (d, J=10 Hz, 1H: NH 1), 8.76 (d, J=9.5 Hz, 1H: NH 6), 11.65 (s, 1H: OH).

[0149] Using the fractions derived from the silica column described above which contain the novel derivative of pristinamycin I_H, 10 mg of 4ζ-methylthio-de(4ζ-dimethylamino)pristinamycin I_H are isolated by means of semi-preparative column chromatography as described above but bringing the proportion of acetonitrile in the eluent phase to 50%.

[0150] NMR spectrum: ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.32 (mt, 1H, 5 β_2), 0.93 (t, J=7.5 Hz, 3H: CH_3 2 γ), from 1.20 to 1.35 (mt, 2H: 3 β_2 and 3 γ_2), 1.30(d, J=7.5 Hz, 3H: CH_3 1 γ), from 1.35 to 2.05 (mt, 9H: 3 γ_1 - 3 β_1 - CH_2 2 β - CH_2 5 δ - CH_2 5γ and 5 β_1), 2.44 (dt, J=13.5 and 1.5 Hz, 1H: 5 ε_2), 2.49 (s,3H: SCH_3), 2.99 (dd, J=12 and 5 Hz, 1H: 4 β_2), 3.09 (dd, J=12.5 and 12 Hz, 1H: 4 β_1), 3.54 and 3.64 (2 mts, 1H each: CH_2 3 δ), 4.17 (dd, J=7 and 6 Hz, 1H: 3 α), 4.49 (broad d, J=13.5 Hz: 1H: 5 ε_1), from 4.70 to 4.80 (mt, 3H: 2 α - 5 α and 4 α), 4.84

(dd, J=10 and 1.5 Hz, 1H: 1 α), 5.51 (d, J=7 Hz, 1H: 6 α), 5.73 (mt, 1H: 1 β), 6.65 (d, J=9.5 Hz, 1H: NH 2), 7.10 (d, J=8 Hz, 2H: 4 δ), 7.22 (d, J=8 Hz, 2H: 4 ε), from 7.20 to 7.40 (mt, 7H: aromatic H 6 = $1'H_4$ and $1'H_5$), 7.87 (d, J=4 Hz, 1H: $1'H_6$), 8.55 (unres.comp., 1H: NH 6), 8.55 (d, J=10 Hz, 1H: NH 1), 11.70 (s, 1H: OH).

[0151] Using the fractions derived from the silica column described above which contain the novel derivative of pristinamycin I, 3 mg of 5γ-hydroxy-4ζ-methylthio-de(4ζ-dimethylamino)pristinamycin I_H are isolated by carrying out semi-preparative column chromatography as described above and maintaining the proportion of acetonitrile in the eluent phase at 45%.

[0152] NMR spectrum: ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): a markedly preponderant isomer is observed: the -OH in the 5 γ position in an axial position. 0.37 (d mt, J=16 Hz, 1H, 5 β_2), 0.93 (t, J=7.5 Hz, 3H: CH_3 2 γ), from 1.20 to 1.45 (mt, 2H: 3 β_2 and 3 γ_2) 1.31 (d, J=7.5 Hz, 3H: CH_3 1 γ), from 1.40 to 1.85 (mt, 5H: 3 γ_1 - CH_2 2 β and CH_2 5 δ), 1.98 (mt, 1H, 3 β_1), 2.17 (d, J=16 Hz, 1H: 5 β_1), 2.50 (s, 3H: SCH_3), 2.77 (dt, J=13.5 and 2 Hz, 1H: 5 ε_2), 2.99 (dd, J=12 and 4 Hz, 1H: 4 β_2), 3.11 (t, J=12 Hz, 1H: 4 β_1), from 3.45 to 3.70 (mt, 2H: CH_2 3 δ), 3.73 (mt, 1H: 5 γ in an equatorial position), 4.13 (t, J=7 Hz, 1H, 3 α), 4.37 (broad d, J=13.5 Hz, 1H: 5 ε_1), from 4,75 to 4.95 (mt, 3H: 2α, 4 α and 5 α), 4.89 (dd, J=10 and 1 Hz, 1H: 1 α), 5.70 (d, J=8 Hz, 1H: 6 α), 5.80 (dq, J=7.5 and 1 Hz, 1H: 1 β), 6.37 (d, J=5 Hz, 1H: NH 4), 6.71 (d, J=10 Hz, 1H: NH 2), 7.10 (d, J=8 Hz, 2H: 4 δ), 7.22 (d, J=8 Hz, 2H: 4 ε), from 7.20 to 7.40 (mt, 5H: aromatic H 6), 7.43 (dd, J=8.5 and 1.5 Hz, 1H: $1'H_4$), 7.46 (dd, J=8.5 and 4 Hz, 1H: $1'H_5$), 7.89 (dd, J=4 and 1.5 Hz, 1H: $1'H_6$), 8.55 (d, J=10 Hz, 1H: NH 1), 9.15 (d, J=8 Hz, 1H: NH 6), 11.70 (s, 1H: OH).

[0153] EXAMPLE 6: Preparation of 4ζ-methyl-de(4ζ-dimethylamino)pristinamycin I_A and of 4ζ-methyl-de(4ζ-dimethylamino)pristinamycin I_H.

[0154] Strain SP92::pVRC508 is cultured in production medium, using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 5 g/l solution of (R,S)-4-methylphenylalanine in 0.1 N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. 49 mg of dry residue are obtained. This residue is taken up in 6 ml of a mixture consisting of 60% water and 40% acetonitrile and injected, in two batches, onto a semi-preparative Nucleosil 7μ C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 44 mg of 4ζ-methyl-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

[0155] NMR spectrum: ^1H (400 MHz, $CDCl_3$ δ in ppm, ref. TMS): 0.52 (dd, J=16 and 6 Hz, 1H, 5 β_2), 0.93 (t, J=7.5 Hz, 3H: CH_3 2 γ), 1.15 (mt, 1H: 3 β_2), from 1.20 to 1.40 (mt, 1H: 3 γ_2), 1.35 (d, J=7.5 Hz, 3H: CH_3 1 γ), from 1.50 to 1.85 (mt, 3H: 3 γ_1 and CH_2 2 β), 2.04 (mt, 1H, 3 β_1), 2.18 (mt, 1H, 5 δ_2), from 2.25 to 2.45 (mt, 2H: 5 δ_1 and 5 β_1), 2.36 (s, 3H: $ArCH_3$),

2.83 (dt, J=13 and 4 Hz, 1H: 5 ε_2), 2.99 (dd, J=13 and 4 Hz, 1H: 4 β_2), 3.28 (s, 3H: $NCH_3$4), 3.31 and 3,59 (2 mts, 1H each: CH_2 3 δ), 3.40 (t, J=13 Hz, 1H: 4 β_1), 4.59 (t, J=7.5 Hz, 1H, 3 α), 4.74 (broad dd, J=13 and 7 Hz, 1H: 5 ε_1), 4.85 (mt, 1H: 2α), 4.89 (broad d, J=10 Hz, 1H: 1α), from 5.25 to 5.35 (mt, 2H: 5 α and 4 α), from 5.85 to 5.95 (mt, 2H: 6 α and 1β), 6.52 (d, J=9.5 Hz, 1H: NH 2), 7.14 (AB limit, J=9 Hz, 4H: 4δ and 4ε), from 7.15 to 7.35 (mt, 5H: aromatic H 6), 7.50 (mt, 2H: 1'H_4 and 1'H_5), 7.81 (dd, J=4 and 2Hz, 1H: 1'H_6), 8.41 (d, J=10 Hz, 1H: NH 1), 8.74 (d, J=9 Hz, 1H: NH 6), 11.63 (s, 1H:OH).

[0156] Using the fractions derived from the silica column described above which contain the new derivative of pristinamycin I_H, 21 mg of 4ζ-methyl-de(4ζ- dimethylamino)pristinamycin I_H (mass spectrometry: $M+H^+$=810) are isolated by carrying out semi-preparative column chromatography as described above.

[0157] **EXAMPLE 7: Preparation of 4ζ-methoxy-de(4ζ-dimethylamino) pristinamycin I_A.**

[0158] Strain SP92::pVRC508 is cultured in production medium using 12 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 5 g/l solution of (RS)-4-methoxyphenylalanine in 0.1 N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 0.35 litres of must recovered from the 12 Erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A

are combined and evaporated. 14 mg of dry residue are obtained. This residue is taken up in 3 ml of a mixture consisting of 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10x250 mm column (Machery Nagel), which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 12 mg of 4ζ-methoxy-de(4ζ- dimethylamino)pristinamycin I_A are obtained.

[0159] NMR spectrum: ^1H (400 MHz, $CDCl_3$, d in ppm, ref. TMS): 0.63 (dd, J=16 and 5.5 Hz, 1H, 5 β_2), 0.96 (t, J=7.5 Hz, 3H: CH_3 2 γ), 1.17 (mt, 1H: 3 β_2), from 1.30 to 1.45 (mt, 1H: 3 γ_2), 1.38 (d, J=7.5 Hz, 3H: CH_3 1 γ) from 1.55 to 1.85 (mt, 3H: 3 γ_1 and CH_2 2 β), 2.05 (mt, 1H, 3 β_1), 2.20 (mt, 1H, 5 δ_2), 2.40 (broad d, J=16 Hz, 1H: 5 δ_1), 2.47 (d, J=16 Hz, 1H: 5 β_1), 2.88 (dt, J=13 and 4 Hz, 1H: 5 ε_2), 2.99 (dd, J=12.5 and 5 Hz, 1H: 4 β_2), 3.30 (s, 3H: NCH_3 4), 3.32 and 3.60 (2 mts, 1H each: CH_2 3 δ), 3.40 (t, J=12.5 Hz, 1H: 4 β_1), 3.80 (s, 3H: OCH_3), 4.60 (t, J=7.5 Hz, 1H, 3 α), 4.80 (broad dd, J=13 and 8.5 Hz, 1H: 5 ε_1), 4.88 (mt, 1H: 2α), 4.92 (broad d, J=10 Hz, 1H: 1α), 5.31 (dd, J=12.5 and 5 Hz, 1H: 4 α), 5.34 (broad d, J=5.5 Hz, 1H: 5 α), 5.90 (d, J=9 Hz, 1H: 6 α), 5.93 (broad q, J=7.5 Hz, 1H: 1β), 6.54 (d, J=9 Hz, 1H: NH 2), 6.87 (d, J=8 Hz, 2H: 4ε), 7.16 (d, J=8 Hz, 2H: 4δ), from 7.15 to 7.40 (mt, 5H: aromatic H 6), 7.50 (mt, 2H: 1'H_5 and 1'H_4), 7.80 (dd, J=4 and 2.5 Ez, 1H: 1'H_6), 8.43 (d, J=10 Hz, 1H: NH 1), 8.78 (d, J=9 Hz, 1H: NH 6), 11.65 (s, 1H:OH).

[0160] **EXAMPLE 8: Preparation of 4ζ-methoxycarbonyl-de(4ζ-dimethylamino) pristinamycin I_A.**

[0161] Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution of (R,S)-4-methoxycarbonylphenylalanine, synthesized as in Example 33, being added at 16 h. At the end of 24 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. 14 mg of dry residue are obtained. This residue is taken up in 3 ml of a mixture consisting of 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 9 mg of 4ζ-methoxycarbonyl- de(4ζ-dimethylamino)pristinamycin I_A are obtained.

[0162] NMR spectrum: ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.70 (dd, J=16 and 6 Hz, 1H, 5 β_2), 0.93 (t, J=7.5 Hz, 3H: CH_3 2 γ), 1.08 (mt, 1H: 3 β_2), from 1.30 to 1.40 (mt, 1H: 3 γ_2), 1.33 (d, J=7.5 Hz, 3H: CH_3 1 γ) from 1.55 to 1.85 (mt, 3H: 3 γ_1, and CH_2 2 β), 2.02 (mt,

1H, 3 β_1), 2.13 (mt, 1H, 5 δ_2), 2.40 (broad d, J=16.5 Hz, 1H: 5 δ_1), 2.48 (d, J=16 Hz, 1H, 5 β_1), 2.89 (dt, J=14.5 and 4.5 Hz, 1H: 5 ε2), 3.10 (dd, J=13.5 and 6 Hz, 1H: 4 β_2), 3.24 (s, 3H: NCH_3 4), 3.38 and 3.61 (2 mts, 1H each: CH_2 3 δ), 3.47 (t, J=13.5 Hz, 1H: 4 β_1), 3.96 (s, 3H: $COOCH_3$), 4.55 (t, J=7.5 Hz, 1H, 3 α), 4.78 (broad dd, J=14.5 and 8 Hz, 1H: 5 ε_1), 4.86 (mt, 1H: 2α), 4.89 (broad d, J=10 Hz, 1H: 1α), 5.33 (broad d, J=6 Hz, 1H: 5 α), 5.42 (dd, J=13.5 and 6 Hz, 1H: 4 α), 5.92 (d, (J=9.5 Hz) and mt, 1H each: 6 α and 1β respectively), 6.52 (d, J=10 Hz, 1H: NH 2), from 7.15 to 7.35 (mt, 5H: aromatic H 6), 7.28 (d, J=8 Hz, 2H: 4δ), 7.43 (dd, J=9 and 1.5 Hz, 1H: 1'H_4), 7.47 (dd, J=9 and 5 Hz, 1H: 1'H_5, 7.66 (d, J=5 and 1.5 Hz, 1H: 1'H_6), 7.98 (d, J=8 Hz, 2H: 4ε), 8.38 (d, J=10 Hz, 1H: NH 1), 8.76 (d, J=9.5 Hz, 1H: NH 6), 11.70 (s, 1H: OH).

[0163] **EXAMPLE 9: Preparation of 4ζ-chloro-de(4ζ-dimethylamino) pristinamycin I_A.**

[0164] Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution of (R,S)-4-chlorophenylalanine in 0.1 N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A

are combined and evaporated. The dry residue is taken up in 3 ml of a mixture consisting of 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 1 mg of 4ζ-chloro-de(4ζ-dimethylamino)pristinamycin I_A is obtained.

[0165] NMR spectrum: ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.93 (t, J=7.5 Hz, 3H: CH_3 2 γ), 0.95 (dd, J=16 and 5 Hz, 1H, 5 β_2), 1.09 (mt, 1H: 3 β_2), from 1.20 to 1.40 (mt, 1H: 3 γ_2), 1.35 (d, J=7.5 Hz, 3H: CH_3 1 γ) from 1.50 to 1.85 (mt, 3H: 3 γ_1 and CH_2 2 β), 2.02 (mt, 1H, 3 β_1), 2.17 (mt, 1H, 5 δ_2), 2.43 (broad d, J=16 Hz, 1H: 5 δ_1), 2.59 (d, J=16 Hz, 1H: 5 β_1), 2.90 (dt, J=13.5 and 4 Hz, 1H: 5 ε_2), 3.04 (dd, J=13 and 6 Hz, 1H: 4 β_2), 3.21 (s, 3H: 4 NCH_3), 3.36 (t, J=13 Hz, 1H: 4 β_1), 3.39 and 3.59 (2 mts, 1H each: CH_2 3 δ), 4.53 (t, J=7.5 Hz, 1H, 3 α), 4.76 (broad dd, J=13.5 and 8 Hz, 1H: 5 ε_1), 4.86 (mt, 1H: 2α), 4.87 (broad d, J=10 Hz, 1H: 1α), 5.38 (mt, 2H: 5 α and 4 α), 5.93 (mt, 2H: 6 α and 1β), 6.52 (d, J=10 Hz, 1H: NH 2), 7.12 (d, J=8 Hz, 2H: 4δ) from 7.15 to 7.35 (mt, 7H: aromatic H 6 and 4ε), 7.38 (dd, J=9 and 4.5 Hz, 1H:1'H_5), 7.43 (broad d, J=9 Hz, 1H: 1'H_4), 7.68 (dd, J=4.5 and 1 Hz, 1H: 1'H_6), 8.36 (d, J=10 Hz, 1H: NH 1), 8.75 (d, J=9 Hz, 1H: NH 6), 11.65 (s, 1H:OH).

[0166] **EXAMPLE 10: Preparation of 4ζ-bromo-de(4ζ-dimethylamino) pristinamycin I_A and of 4ζ-bromo-de(4ζ-dimethylamino)pristinamycin I_H.**

[0167] Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution of (R,S)-4-bromophenylalanine

in 0.1 N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. The dry residue is taken up in 6 ml of a mixture consisting of 60% water and 40% acetonitrile and injected in two batches onto a semi-preparative Nucleosil 7μ C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 6 mg of 4ζ-bromo-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

[0168] NMR spectrum: ^{1}H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.93 (J=7.5 Hz, 3H: CH_3 2 γ), 0.95 (dd, J=16 and 5 Hz, 1H, 5 β_2), 1.10 (mt, 1H: 3 β_2), 1.35 (d, J=7.5 Hz, 3H: CH_3 1 γ) 1.36 (mt, 1H: 3 γ_2), from 1.50 to 1.85 (mt, 3H, 3 γ_1 and CH_2 2 β), 2.02 (mt, 1H, 3 β_1), 2.18 (mt, 1H: 5 δ_2), 2.43 (broad d, J=16 Hz, 1H: 5 δ_1), 2.59 (d, J=16 Hz, 1H: 5 β_1), 2.90 (dt, J=13 and 4 Hz, 1H: 5 ε_2), 3.02 (dd, J=13 and 5.5 Hz, 1H: 4 β_2), 3.21 (s, 3H: 4 NCH_3), 3.33 (dd, J=13-11 Hz, 1H: 4 β_1), 3.39 and 3.59 (2 mts, 1H each: CH_2 3 δ), 4.53 (t, J=7.5 Hz, 1H, 3 α), 4.76 (broad dd, J=13 and 7 Hz, 1H: 5 ε_1), 4.86 (mt, 1H, 2α), 4.89 (d broad, J=10 Hz, 1H: 1α), 5.37 (broad d,

J=5 Hz, 1H: 5 α), (dd, J=11 and 5.5 Hz, 1H: 4 α), 5.92 (mt, 2H: 6 α and 1β), 6.56 (d, J=9.5 Hz, 1H: NH 2), 7.08 (d, J=8 Hz, 2H: 4δ), from 7.15 to 7.35 (mt, 5H: aromatic H 6), 7.40 (mt, 4H: $1'H_4$ - $1'H_5$ and 4ε, 7.70 (broad d, J=5 Hz, 1H: $1'H_6$), 8.40 (d, J=10 Hz, 1H: NH 1), 8.77 (d, J=9 Hz, 1H: NH 6), 11.68 (s, 1H: OH).

[0169] Using the fractions derived from the silica column described above which contain the new derivative of pristinamycin I_H, 3 mg of 4ζ-bromo-de(4ζ-dimethylamino)pristinamycin I_H (mass spectrometry: $M+H^+=874$) are isolated by carrying out semi-preparative column chromatography as described above.

[0170] **EXAMPLE 11: Preparation of 4ζ-iodo-de(4ζ-dimethylamino)pristinamycin I_A and of 4ζ-iodo-de(4ζ-dimethylamino)pristinamycin I_H.**

[0171] Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution of (RS)-4-iodophenylalanine in sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried on sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. The dry residue is taken up in 6 ml of a mixture consisting of 60% water and 40% acetonitrile and injected in two batches onto a semi-preparative Nucleosil 7μ C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 60%

100 mM phosphate buffer, pH 2.9, and 40% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 12 mg of 4ζ-iodo-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

[0172] NMR spectrum: ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.93 (J=7.5 Hz, 3H: CH_3 2 γ), 0.95 (dd, J=16 and 5.5 Hz, 1H: 5 β_2), 1.10 (mt, 1H: 3 β_2), 1.35 (d, J=7.5 Hz, 3H: CH_3 1 γ), 1.38 (mt, 1H: 3 γ_2), from 1.55 to 1.85 (mt, 3H, 3 γ_1 and CH_2 2 β), 2.02 (mt, 1H, 3 β_1), 2.17 (mt, 1H: 5 δ_2), 2.43 (broad d, J=16.5 Hz, 1H: 5 δ_1), 2.60 (d, J=16 Hz, 1H: 5 β_1), 2.89 (dt, J=14 and 4.5 Hz, 1H: 5 ε_2), 3.02 (dd, J=13 and 5.5 Hz, 1H: 4 β_2), 3.21 (s, 3H: NCH_3 4), 3.31 (dd, J=13 and 11 Hz, 1H: 4 β_1), 3.39 and 3.59 (2 mts, 1H each: CH_2 3 δ), 4.53 (t, J=7.5 Hz, 1H, 3 α), 4.75 (broad dd, J=14 and 8 Hz, 1H: 5 ε_1), 4.83 (mt, 1H: 2α), 4.88 (broad d, J=10 Hz, 1H: 1α), 5.37 (broad d, J=5.5 Hz, 1H: 5 α), 5.39 (dd, J=11 and 5.5 Hz, 1H: 4 α), 5.92 (mt, 2H: 6 α and 1β), 6.54 (d, J=9.5 Hz, 1H: NH 2), 6.94 (d, J=7.5 Hz, 2H: 4δ), from 7.15 to 7.50 (mt, 5H: aromatic H 6), 7.36 (dd, J=9 and 4 Hz, 1H: 1'H_5), 7.43 (broad d, J=9 Hz, 1H: 1'H_4), 7.62 (d, J=7.5 Hz, 2H: 4ε), 7.68 (d, J=4 Hz, 1H: 1'H_6), 8.38 (d, J=10 Hz, 1H: NH 1), 8.76 (d, J=9 Hz, 1H: NH 6), 11.60 (s, 1H: OH).

[0173] Using the fractions derived from the silica column described above which contain the new derivative of pristinamycin I_H, 6 mg of 4ζ-iodo-de(4ζ-dimethylamino)pristinamycin I_H (mass spectrometry: $M+H^+$=922) are isolated by carrying out semi-preparative column chromatography as described above.

[0174] **EXAMPLE 12 Preparation of 4ζ-trifluoromethyl-de(4ζ-dimethylamino) pristinamycin I_A and of 4ζ-trifluoromethyl-de(4ζ-dimethylamino)pristinamycin I_H.**

[0175] Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 5 g/l solution of (S)-4-trifluoromethylphenylalanine in 0.1 N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried on sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. The dry residue is taken up in 3 ml of a mixture consisting of 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 5 mg of 4ζ-trifluoromethyl-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

[0176] NMR spectrum: ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.86 (dd, J=16 and 5.5 Hz, 1H, 5 β_2), 0.91 (t, J=7.5 Hz, 3H: CH_3 2γ), 1.13 (mt, 1H: 3 β_2), 1.31 (d, J=7.5 Hz, 3H: CH_3 1 γ) 1.42 (mt, 1H: 3 γ_2), from 1.55 to 1.80 (mt, 3H: 3 γ_1 and CH_2 2 β), 2.02 (mt, 1H, 3 β_1), 2.15 (mt, 1H, 5 δ_2), 2.40 (broad d, J=16.5 Hz, 1H: 5 δ_1), 2.55 (d, J=16 Hz, 1H: 5 β_1), 2.88 (dt,

J=14 and 4 Hz, 1H: 5 ε_2), 3.18 (s, 3H: NCH_3 4), 3.20 and 3.31 (2 dd, respectively J=13 and 6 Hz and J=13 and 10 Hz, 1H each: 4 β_2 and 4 β_1), 3.42 and 3.60 (2 mts, 1H each: CH_2 3 δ), 4.50 (t, J=7.5 Hz, 1H, 3 α), 4.73 (broad dd, J=14 and 7.5 Hz, 1H: 5 ε_1), 4.83 (mt, 1H: 2α), 4.91 (broad d, J=10 Hz, 1H: 1α), 5.40 (broad d, J=5.5 Hz, 1H: 5 α), 5.55 (dd, J=10 and 6 Hz, 1H: 4 α), 5.87 (d, J=9.5 Hz, 1H: 6 α), 5.90 (broad q, J=7.5 Hz, 1H: 1β), 6.68 (d, J=9.5 Hz, 1H: NH 2), from 7.15 to 7.40 (mt, 9H: 4δ-aromatic H 6 - 1'H_5 and 1'H_4), 7.52 (d, J=8 Hz, 2H: 4ε), 7.68 (d, J=4 and 1.5 Hz, 1H: 1'H_6), 8.43 (d, J=10 Hz, 1H: NH 1), 8.76 (d, J=9.5 Hz, 1H: NH 6), 11.70 (s, 1H: OH).

[0177] Using the fractions derived from the silica column described above which contain the new derivative of pristinamycin I_H, 4 mg of ζ-trifluoromethyl-de(4ζ-dimethylamino)pristinamycin I_H (mass spectrometry: $M+H^+$=864) are isolated by carrying out semi-preparative column chromatography as described above.

[0178] **EXAMPLE 13: Preparation of 4ζ-tert-butyl-de(4ζ-dimethylamino) pristinamycin I_A.**

[0179] Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 5 g/l solution of (R,S)-4-tert-butylphenylalanine, synthesized as in Example 35-1, in 0.1 N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyers are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried on sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The

fractions containing the new derivative of pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% water and 40% acetonitrile and injected in 2 batches onto a semi-preparative Nucleosil 7μ C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 30 mg of 4ζ-tert-butyl-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

[0180] NMR spectrum: ^{1}H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS, ref. TMS): 0.21 (dd, J=16 and 5.5 Hz, 1H, 5 β_2), 0.91 (t, J=7.5 Hz, 3H: CH_3 2 γ), 1.17 (mt, 1H: 3 β_2), from 1.20 to 1.40 (mt, 1H: 3 γ_2), 1.33 (s, 9H: CH_3 of tert-butyl), 1.35 (d, J=7.5 Hz, 3H: CH_3 1 γ), from 1.50 to 1.85 (mt, 3H: 3 γ_1 and CH_2 2 β), 2.04 (mt, 1H, 3 β_1), 2.13 (mt, 1H, 5 δ_2), 2.30 (mt, 2H: 5 δ_1 and 5 δ_1), 2.80 (dt, J=13 and 4 Hz, 1H: 5 ε_2), 3.00 (dd, J=12 and 4 Hz, 1H: 4 β_2), 3.29 (s, 3H: $NCH_3$4), 3.31 and 3.59 (2 mts, 1H each: CH_2 3 δ), 3.40 (t, J=12 Hz, 1H: 4 β_1), 4.57 (t, J=7.5 Hz, 1H, 3 α), 4.74 (broad dd, J=13 and 7 Hz, 1H: 5 ε_1), 4.85 (mt, 1H: 2α), 4.90 (broad d, J=10 Hz, 1H: 1α), 5.21 (broad d, J=5.5 Hz, 1H: 5 α), 5.25 (dd, J=12 and 4 Hz, 1H: 4 α), 5.87(d, J=9 Hz, 1H: 6 α), 5.92 (broad q, J=7.5 Hz, 1H: 1 [lacuna] 1H: 1'H_6), 8.45 (d, J=10 Hz, 1H: NH 1), 8.74 (d, J=9 Hz, 1H: NH 6), 11.65 (s, 1H:OH).

[0181] EXAMPLE 14: Preparation of 4ζ-isopropyl-de(4ζ-dimethylamino) pristinamycin I_A and of 4ζ-isopropyl-de(4ζ-dimethylamino)pristinamycin I_E.

[0182] Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution of (R,S)-4-

isopropylphenylalanine, synthesized as in Example 36-1, in 0.1 N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. 61 mg of the dry residue are obtained. This residue is taken up in 9 ml of a mixture consisting of 60% water and 40% acetonitrile and injected in 3 batches onto a semi-preparative Nucleosil 7μ C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 51 mg of 4ζ-isopropyl-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

[0183] NMR spectrum: ^{1}H (250 MHz, $CDCl_3$, δ in ppm, ref. TMS, ref. TMS): 0.31 (dd, J=16 and 5.5 Hz, 1H, 5 β_2), 0.91 (t, J=7.5 Hz, 3H: CH_3 2 γ), from 1.00 to 1.45 (mt, 2H: 3 β_2 and 3 γ_2), 1.25 (d, J=7.5 Hz, 6H: CH_3 of isopropyl), 1.35 (d, J=7.5 Hz, 3H: CH_3 1 γ), from 1.50 to 1.85 (mt, 3H: 3 γ_1 and CH_2 2 β), from 1.95 to 2.20 (mt, 2H, 3 β_1 and 5 δ_2), 2.30 (mt, 2H: 5 δ_1 and 5 β_1), 2.80 (dt, J=13 and 4 Hz, 1H: 5 ε_2), 2.88 (mt, 1H: CH of isopropyl), 2.98 (dd, J=12 and 4 Hz, 1H: 4 β_2), 3.30 (s, 3H: NCH_3 4), 3.32 and 3.55 (2 mts, 1H each: CH_2 3 δ), 3.38 (t, J=12 Hz, 1H: 4 β_1), 4.55 (t, J=7.5 Hz, 1H, 3 α), 4.72 (broad dd, J=13 and 7 Hz, 1H: 5 ε_1), 4.85 (mt, 1H:

2α), 4.88 (broad d, J=10 Hz, 1H: 1α), 5.21 (broad d, J=5.5 Hz, 1H: 5α), 5.25 (dd, J=12 and 4 Hz, 1H: 4 α), 5.87 (d, J=9 Hz, 1H: 6 α), 5.90 (broad q, J=7.5 Hz, 1H: 1 β), 6.50 (d, J=9.5 Hz, 1H: NH 2), from 7.05 to 7.35 (mt, 9H: aromatic H 6 - 4ε and 4δ), 7.50 (mt, 2H: $1'H_5$ and $1'H_4$), 7.86 (dd, J=4 and 1.5 Hz, 1H: $1'H_6$), 8.40 (d, J=10 Hz, 1H: NH 1), 8.72 (d, J=9 Hz, 1H: NH 6), 11.60 (s, 1H: OH).

[0184] Using the same fractions derived from the silica column described above, which fractions also contain the new derivative of pristinamycin I_E, 5 mg of ζ-isopropyl-de(4ζ-dimethylamino)pristinamycin I_E are isolated by carrying out semi-preparative column chromatography as described above.

[0185] NMR spectrum: ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.20 (mt, 1H, 5 β_2), 0.92 (t, J=7.5 Hz, 3H: CH_3 2 γ), from 1.15 to 1.40 (mt, 2H: 3 β_2 and 3 γ_2), 1.24 (d, J=7.5 Hz, 6H: CH_3 of isopropyl), 1.34 (d, J=7.5 Hz, 3H: CH_3 1 γ), from 1.35 to 2.05 (mt, 9H: 3 γ_1 - 3 β_1 - CH_2 2 β - CH_2 5 δ - CH_2 5 γ and 5 β_1), 2.45 (dt, J=13 and 1.5 Hz, 1H: 5 ε_2), 2.89 (mt, 1H: ArCH), 3.09 (dd, J=14 and 7Hz, 1H: 4 β_2), 3.17 (s, 3H: NCH_3 4), 3.25 (dd, J=14 and 9 Hz, 1H: 4 β_1), 3.32 and 3.52 (2 mts, 1H each: CH_2 3 δ), 4.55 (mt, 2H: 3 α and 5 ε_1), 4.80 (mt, 1H: 2α), 4.89 (dd, J=10 and 1.5 Hz, 1H: 1α), 4.90 (mt, 1H: 5 α), 5.35 (dd, J=9 and 7Hz, 1H: 4 α), 5.60 (d, J=8 Hz, 1H: 6 α), 5.89 (dq, J=7.5 and 1.5 Hz, 1H: 1β), 6.65 (d, J=9.5 Hz, 1H: NH 2), 7.08 (d, J=8 Hz, 2H: 4δ), 7.14 (d, J=8 Hz, 2H: 4ε), from 7.20 to 7.40 (mt, 7H: aromatic H 6 - $1'H_4$ and $1'H_5$), 7.77 (broad d, J=4 Hz, 1H: $1'H_6$), 8.46 (d, J=10 Hz, 1H: NH1), 8.48 (d, J=8 Hz, 1H: NH 6), 11.70 (s, 1H: OH).

[0186] **EXAMPLE 15: Preparation of 4ε-methylamino-de(4ζ-dimethylamino) pristinamycin I_A.**

[0187] Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution of (R,S)-3-methylaminophenylalanine, synthesized as in Example 35-3, in water being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% of 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. 19 mg of dry residue are obtained. This residue is taken up in 3 ml of a mixture consisting of 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 8 mg of 4ε-methylamino-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

[0188] NMR spectrum: ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.93 (t, J=7.5 Hz, 3H: CH_3 2 γ), 1.00 (dd, J=16 and 6Hz, 1H, β_2), 1.17 (mt, 1H: 3 β_2), from 1.25 to 1.40 (mt, 2H: 3 γ_2), 1.35 (d, J=7.5 Hz, 3H: CH_3 1 γ), from 1.55 to 1.80 (mt, 3H: 3 γ_1 and CH_2 2 β), 2.03 (mt, 1H,

3 β_1), 2.23 (mt, 1H, 5 δ_2), 2.39 (broad d, J=16 Hz, 1H: 5 δ_1), 2.52 (d, J=16 Hz, 1H: 5 β_1), 2.78 (s, 3H: $ArNCH_3$ 4), 2.85 (dt, J=13 and 4 Hz, 1H: 5 ε_2), 2.99 (dd, J=13 and 4.5 Hz, 1H: 4 β_2), 3.23 (s, 3H: NCH_3 4), 3.25 (t, J=13 Hz, 1H: 4β_1), 3.38 and 3.58 (2mts, 1H each: CH_2 3 δ), 4.05 (unres. comp., 1H: ArNH), 4.58 (dd, J=6.5 and 7.5 Hz, 1H, 3 α), 4.76 (broad dd, J=13 and 8 Hz, 1H: 5 ε_1), 4.85 (mt, 1H: 2α), 4.87 (broad d, J=10 Hz, 1H: 1α), 5.35 (dd, J=13 and 4.5 Hz, 1H: 4 α), 5.38 (broad d, J=6 Hz, 1H: 5 α), 5.90 (d, J=9.5 Hz, 1H: 6 α), 5.91 (mt, 1H: 1β), 6.36 (broad s, 1H: H 2 of the aromatic moiety at position 4), from 6.45 to 6.55 (mt, 2H: H4 and H6 of the aromatic moiety in position 4), 6.53 (d, J=10 Hz, 1H: NH 2), 7.12 (t, J=8 Hz, 1H: H 5 of the aromatic moiety in position 4), from 7.15 to 7.45 (mt, 5H: aromatic H 6), 7.35 (mt, 2H: 1'H_4 and 1'H_5), 7.75 (t, J=3 Hz, 1H: 1'H_6), 8.40 (d, J=10 Hz, 1H: NH 1), 8.78 (d, J=9.5 Hz, 1H: NH 6), 11.60 (s, 1H: OH).

[0189] EXAMPLE 16: Preparation of 4ε-methoxy-de(4ζ-dimethylamino) pristinamycin I_A and of 4ε-methoxy-de(4ζ-dimethylamino)pristinamycin I_H.

[0190] Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Examle 3, with 1 ml of a 5 g/l solution of (S)-3-methoxyphenylalanine in 0.1N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A

are combined and evaporated. 41 mg of dry residue are obtained. This residue is taken up in 6 ml of a mixture consisting of 60% water and 40% acetonitrile and injected in 2 batches onto a semi-preparative Nucleosil 7μ C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 28 mg of 4ε-methoxy-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

[0191] NMR spectrum: ^{1}H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.52 (dd, J=16 and 5.5 Hz, 1H, 5 β_2), 0.90 (t, J=7.5 Hz, 3H: CH_3 2 γ), from 1.10 to 1.34 (mt, 2H: 3 β_2 and 3 γ_2), 1.34 (d, J=7.5 Hz, 3H: CH_3 1 γ), from 1.50 to 1.80 (mt, 3H: 3 γ_1 and CH_2 2 β), 2.40 (mt, 1H, 3 β_1), 2.20 (mt, 1H, 5 δ_2), 2.35 (broad d, J=16 Hz, 1H: 5 δ_1), 2.38 (d, J=16 Hz, 1H: 5 β_1), 2.83 (dt, J=13 and 4 Hz, 1H: 5 ε_2), 2.97 (dd, J=12 and 4 Hz, 1H: 4 β_2), 3.28 (s, 3H: NCH_3 4), 3.28 and 3.56 (2 mts, 1H each: CH_2 3 δ), 3.40 (t, J=12 Hz, 1H: 4 β_1), 3.80 (s, 3H: OCH_3), 4.58 (t, J=7.5 Hz, 1H, 3 α), 4.76 (broad dd, J=13 and 8 Hz, 1H: 5 ε_1), 4.85 (mt, 1H: 2α), 4.90 (broad d, J=10 Hz, 1H: 1α): 5.27 (dd, J=12 and 4 Hz, 1H: 4α), 5.30 (broad d, J=5.5 Hz, 1H; 5α), 5.89 (d, J=9.5 Hz, 1H: 6 α), 5.91 (broad q, J=7.5 Hz, 1H: 1β), 6.51 (d, J=10 Hz, 1H: NH 2), from 6.80 to 6.90 (mt, 3H: H 2 - H 4 and H 6 of the aromatic moiety in position 4), from 7.15 to 7.40 (mt, 6H: H 5 of the aromatic moiety in position 4 and aromatic H 6), 7.45 (broad d, J=9 Hz, 1H: 1'H_4), 7.50 (dd, J=9 and 4 Hz, 1H:1'H_5), 7.80 (broad d, J = 4 Hz, 1H: 1'H_6), 8.40 (d, J=10 Hz, 1H: NH 1), 8.73 (d, J=9.5 Hz, 1H: NH 6), 11.62 (s, 1H: OH).

[0192] Using the fractions derived from the silica column described above which contain the new derivative of pristinamycin I_H, 7 mg of 4ε-methoxy-de(4ζ-dimethylamino)pristinamycin

I_H (mass spectrometry: $M+H^+ = 826$) are isolated by carrying out semi-preparative column chromatography as described above.

[0193] **EXAMPLE 17: Preparation of 4ε-fluoro-4ζ-methyl-de(4ζ-dimethylamino) pristinamycin I_A.**

[0194] Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution of (R,S)-3-fluoro-4-methylphenylalanine, synethesized as in Example 34-5, in 0.1N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. 15 mg of dry residue are obtained. This residue is taken up in 3 ml of a mixture consisting of 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10x250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted witih one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 9 mg of 4ϵ-fluoro-4ζ-methyl-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

[0195] NMR spectrum: ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.60 (dd, J=16 and 5.5 Hz, 1H, 5 β_2), 0.91 (t, J=7.5 Hz, 3H: CH_3 2 γ), 1.12 (mt, 1H: 3 β_2), from 1.25 to 1.35 (mt, 1H: 3γ_2), 1.33 (d, J=7.5 Hz, 3H: CH_3 1 γ), from 1.50 to 1.85 (mt, 3H: 3 γ_1 and CH_2 2 β), 2.02 (mt, 1H, 3 β_1), 2.13 (mt, 1H, 5 δ_2), 2.27 (s, 3H: $ArCH_3$), 2.36 (broad d, J=16 Hz, 1H: 5 δ_1), 2.45 (d, J=16 Hz, 1H: 5 β_1), 2,85 (dt, J=13 and 4.5 Hz, 1H: 5 ε_2), 2.97 (dd, J=12.5 and 4.5 Hz, 1H: 4 β_2), 3.23 (s, 3H: NCH_3 4), 3.30 and 3.56 (2 mts, 1H each: CH_2 3 δ), 3.37 (t, J=12.5 Hz, 1H: 4 β_1), 4.55 (t, J=7.5 Hz, 1H, 3 α), 4.75 (broad dd, J=13 and 8 Hz, 1H: 5 ε_1), 4.83 (mt, 1H: 2α), 4.89 (broad d, J=10 Hz, 1H: 1α), 5.29 (dd, J=12.5 and 4.5 Hz, 1H: 4 α), 5.32 (broad d, J=5.5 Hz, 1H: 5 α), 5.89 (d J=9.5 Hz, 1H: 6 α), 5.92 (mt, 1H: 1β), 6.49 (d, J=10 Hz, 1H: NH 2), 6.90 (mt, 2H: H 2 and H 6 of the aromatic moiety in position 4), 7.11 (t, J=8 Hz, 1H: H 5 of the aromatic moiety in position 4), from 7.10 to 7.30 (mt, 5H: aromatic H 6), 7.43 (dd, J=8.5 and 1 Hz, 1H: 1'H_4), 7.49 (dd, J=8.5 and 4.5 Hz, 1H: 1'H_5), 7.75 (dd, J=4.5 and 1 Hz, 1H: 1'H_6), 8.48 (d, J=10 Hz, 1H: NH 1), 8.70 (d, J=9.5 Hz, 1H: NH 6), 11.60 (s, 1H: OH).

[0196] EXAMPLE 18: Preparation of 4ζ-ethylamino-de(4ζ-dimethylamino) pristinamycin I_A

[0197] Strain SP92::pVRC508 is cultured in production medium using 50 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-4-ethylaminophenylalanine dihydrochloride, synthesized as in Example 33, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 1.5 litres of must recovered from the 50 erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated.

The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica column (30 g) which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4ζ-ethylamino-de(4ζ-dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 65% water and 35% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10x250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% acetonitrile. The fractions containing 4ζ-ethylamino-de(4ζ-dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 10 mg of 4ζ-ethylamino-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

[0198] NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.72 (dd, J = 16 and 6 Hz, 1H: 1H of the CH_2 in 5 β); 0.90 (t, J = 7.5 Hz, 3H: CH_3 in 2γ); 1.15 (mt, 1H: 1H of the CH_2 in 3 β); from 1.20 to 1.40 (mt, 1H: 1H of the CH_2 in 3 γ); 1.27 (t, J = 7.5 Hz, 3H: CH_3 of the ethyl); 1.33 (d, J = 7 Hz, 3H: CH_3 in 1 γ); from 1.50 to 1.65 (mt, 1H: the other H of the CH_2 in 3 γ); 1.60 and 1.74 (2 mts, 1H each: CH_2 in 2 β); 2.02 (mt, 1H: the other H of CH_2 in 3 β); 2.21 and 2.33 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ); 2.40 (d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.82 (dt, J = 13 and 4.5 Hz, 1H: 1H of the CH_2 in 5ε); 2.89 (dd, J = 12 and 4 Hz, 1H: 1H of the CH_2 in 4 β); 3.10 (mt, 2H: NCH_2 of the ethyl); from 3.20 to 3.35 (mt, 1H: 1H of the CH_2 in 3 δ); 3.26 (s, 3H: NCH_3); 3.31 (t, J = 12 Hz, 1H: the other H of the CH_2 in 4 β); 3.54 (mt, 1H: the other H of the CH_2 in 3 δ); 3.67 (unres. comp., 1H: NH); 4.56 (dd, J = 6.5 and 7 Hz, 1H: 3 α); 4.75 (broad dd, J = 13 and 8 Hz, 1H: the other H of the CH_2 in 5 ε); 4.84 (mt, 1H: 2 α); 4.90 (broad d, J = 10 Hz, 1H : 1 α); 5.24 (dd, J = 12 and 4 Hz, 1H: 4 α);

5.32 (broad d, J = 6 Hz, 1H: 5 α); 5.88 (d, J = 9.5 Hz, 1H : 6 α); 5.90 (mt, 1H : 1 β); 6.52 (d, J = 8 Hz, 3H : NH in 2 and aromatic H in 4 ε); 7.00 (d, J = 8 Hz, 2H : aromatic H in 4 δ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.46 (limiting AB, 2H: $1'H_4$ and $1'H_5$); 7.84 (dd, J = 4 and 1 Hz, 1H: $1'H_6$); 8.45 (d, J = 10 Hz, 1H: NH in 1); 8.77 (d, J = 9.5 Hz, 1H: NH in 6); 11.65 (s, 1H: OH).

[0199] **EXAMPLE 19: Preparation of 4ζ-diethylamino-de(4ζ-dimethylamino) pristinamycin I_A**

[0200] Strain SP92::pVRC508 is cultured in production medium using 50 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-4-ethylaminophenylalanine dihydrochloride, synthesized as in Example 33, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 1.5 litres of must recovered from the 50 erlenmeyer flasks are extracted with 2 volumes of a mixture of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica column (30 g) which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4ζ-diethylamino-de(4ζ-dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% water and 40% acetonitrile and injected in two portions onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 68% 100 mM phosphate buffer, pH 2.9, and 32% acetonitrile. The fractions containing 4ζ-diethylamino-de(4ζ-dimethylamino)pristinamycin I_A are combined and extracted with one

volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 50 mg of 4ζ-diethylamino-de(4ζ-dimethylamino) pristinamycin I_A are obtained.

[0201] NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.65 (dd, J = 16 and 6 Hz, 1H: 1H of the CH_2 in 5 β); 0.90 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); 1.14 (t, J = 7 Hz, 6H: CH_3 of the ethyl); 1.15 (mt, 1H: 1H of the CH_2 in 3 β); 1.26 (mt, 1H: 1H of the CH_2 in 3 γ); 1.32 (d, J = 6.5 Hz, 3H: CH_3 in 1 γ); 1.55 (mt, 1H: the other H of the CH_2 in 3 γ); 1.63 and 1.75 (2 mts, 1H each: CH_2 in 2 β): 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.22 and 2.31 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ); 2.37(d, J=16 Hz, 1H: the other H of the CH_2 in 5 β); 2.80 (dt, J = 13 and 4.5 Hz, 1H: 1H of the CH_2 in 5 ε); 2.89 (dd, J = 12.5 and 4 Hz, 1H : 1H of the CH_2 in 4 β); from 3.20 to 3.40 (mt, 6H: NCH_2 of the ethyl - 1H of the CH_2 in 3 δ and the other H of the CH_2 in 4 β); 3.27 (s, 3H: NCH_3); 3.55 (mt, 1H: the other H of the CH_2 in 3 δ); 4.58 (dd, J = 8 and 6 Hz, 1H: 3 α); 4.76 (broad dd, J = 13 and 7.5 Hz, 1H: the other H of the CH_2 in 5 ε); 4.84 (mt, 1H: 2 α); 4.89 (dd, J = 10 and 1 Hz, 1H: 1 α); 5.21 (dd, J = 12.5 and 4 Hz, 1H: 4 α); 5.28 (broad d, J = 6 Hz, 1H : 5 α); 5.87 (d, J = 9.5 Hz, 1H: 6 α); 5.90 (mt, 1H: 1 β); 6.52 (d, J = 9.5 Hz, 1H : NH in 2); 6.60 (d, J = 8 Hz, 2H: aromatic H in 4 ε); 7.02 (d, J = 8 Hz, 2H: aromatic H in 4 δ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.46 (limiting AB, 2H: $1'H_4$ and $1'H_5$); 7.88 (dd, J = 4.5 and 2.5 Hz, 1H: $1'H_6$); 8.43 (d, J = 10 Hz, 1H: NH in 1); 8.76 (d, J = 9.5 Hz, 1H: NH in 6); 11.62 (s, 1H: OH).

[0202] EXAMPLE 20: Preparation of 4ζ-diallylamino-de(4ζ-dimethylamino) pristinamycin I_A

[0203] Strain SP92::pVRC508 is cultured in production medium using 94 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-4-

diallylaminophenylalanine dihydrochloride, synthesized as in Example 38-1, in water being added at 16h. At the end of 40h of culture, the 2.8 litres of must recovered from the 94 erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4ζ-diallylamino-de(4ζ-dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Machery Nagel) column, which is eluted with a mixture consisting of 52% 100 mM phosphate buffer, pH 2.9, and 48% acetonitrile. The fractions containing 4ζ-diallylamino-de(4ζ-dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 15 mg of 4ζ-diallylamino-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

[0204] NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.55 (dd, J = 16 and 6 Hz, 1H : 1H of the CH_2 in 5 β); 0.93 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); 1.18 (mt, 1H: 1H of the CH_2 in 3 β); 1.25 (mt, 1H : 1H of the CH_2 in 3 γ); 1.34 (d, J = 6.5 Hz, 3H: CH_3 in 1 γ); 1.59 (mt, 1H: the other H of the CH_2 in 3 γ); 1.68 and 1.78 (2 mts, 1H each: CH_2 in 2 β); 2.04 (mt, 1H: the other H of CH_2 in 3 β); 2.25 and 2.34 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ); 2.40 (d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.83 (dt, J = 13 and 4.5 Hz, 1H: 1H of the CH_2 in 5 ε); 2.92 (dd, J = 12 and 4 Hz, 1H: 1H of the CH_2 in 4 β); from 3.20 to 3.30

(mt, 1H: 1H of the CH_2 in 3 δ); 3.29 (s, 3H: NCH_3); 3.33 (t, J = 12 Hz, 1H: the other H of the CH_2 in 4 β); 3.57 (mt, 1H: the other H of the CH_2 in 3 δ); 3.93 (limiting AB, 4H: NCH_2 of the allyl); 4.60 (dd, J = 8 and 6.5 Hz, 1H: 3 α); 4.78 (broad dd, J = 13 and 7.5 Hz, 1H: the other H of the CH_2 in 5 ε); 4.87 (mt, 1H: 2 α); 4.92 (dd, J = 10 and 1 Hz, 1H: 1 α); from 5.10 to 5.25 (mt, 5H: 4 α and $=CH_2$ of the allyl); 5.28 (broad d, J = 6 Hz, 1H: 5 α); 5.85 (mt, 2H: CH= of the allyl); 5.92 (d, J = 9.5 Hz, 1H: 6 α); 5.94 (mt, 1H : 1 β); 6.54 (d, J = 10 Hz, 1H: NH in 2); 6.65 (d, J = 8 Hz, 2H : aromatic H in 4 ε); 7.05 (d, J = 8 Hz, 2H: aromatic H in 4 δ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.51 (limiting AB, 2H: $1'H_4$ and $1'H_5$); 7.88 (dd, J = 4 and 2 Hz, 1H: $1'H_6$); 8.43 (d, J = 10 Hz, 1H: NH in 1); 8.77 (d, J = 9.5 Hz, 1H: NH in 6); 11.65 (s, 1H : OH).

[0205] EXAMPLE 21: Preparation of 4ζ-allylethylamino-de(4ζ-dimethylamino) pristinamycin I_A

[0206] Strain SP92::pVRC508 is cultured in production medium using 26 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-4-allylethylaminophenylalanine dihydrochloride, synthesized as in Example 39-4, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 0.78 litre of must recovered from the 26 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4ζ-allylethylamino-de(4ζ-dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7

ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 52% 100 mM phosphate buffer, pH 2.9, and 48% of acetonitrile. The fractions containing 4ζ-allylethylamino-de(4ζ-dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 20 mg of 4ζ-allylethylamino-de(4ζ-dimethylamino) pristinamycin I_A are obtained.

[0207] NMR spectrum. ^{1}H (400 MHz, CDCl3, δ in ppm, ref. TMS): 0.58 (dd, J = 16 and 6 Hz, 1H: 1H of CH_2 in 5 β); 0.91 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); 1.16 (t, J = 7 Hz, 3H: CH_3 of the ethyl); 1.16 (mt, 1H: 1H of the CH_2 in 3 β); 1.25 (mt, 1H: 1H of CH_2 in 3 γ); 1.32 (d, J = 6.5 Hz, 3H: CH_3 in 1 γ); 1.54 (mt, 1H: the other H of the CH_2 in 3 γ); 1.63 and 1.75 (2 mts, 1H each: CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.23 and 2.31 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ); 2.37 (d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.80 (dt, J = 13 and 4.5 Hz, 1H : 1H of CH_2 in 5 ε); 2.87 (dd, J = 12 and 4 Hz, 1H: 1H of the CH_2 in 4 β); from 3.15 to 3.30 (mt, 1H: 1H of the CH_2 in 3 δ); 3.26 (s, 3H: NCH_3); 3.30 (t, J = 12 Hz, 1H: the other H of CH_2 in 4 β); 3.36 (mt, 2H: NCH_2 of the ethyl); 3.54 (mt, 1H: the other H of the CH_2 in 3 δ); 3.90 (limiting AB, 2H: NCH_2 of the allyl); 4.57 (dd, J = 8 and 6 Hz, 1H: 3 α); 4.76 (broad dd, J = 13 and 7.5 Hz, 1H: the other H of the CH_2 in 5 ε); 4.84 (mt, 1H: 2 α); 4.89 (dd, J = 10 and 1 Hz, 1H: 1 α); from 5.05 to 5.20 (mt, 3H: 4 α and =CH_2 of the allyl); 5.27 (broad d, J = 6 Hz, 1H : 5 α); 5.83 (mt, 1H: CH= of the allyl); 5.88 (d, J = 9.5 Hz, 1H: 6 α); 5.91 (mt, 1H: 1 β); 6.50 (d, J = 10 Hz, 1H: NH in 2); 6.60 (d, J = 8 Hz, 2H: aromatic H in 4 ε); 7.02 (d, J = 8 Hz, 2H: aromatic H in 4 δ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.47 (limiting AB,

2H: 1'H_4 and 1'H_5); 7.88 (dd, J = 4 and 2 Hz, 1H: 1'H_6); 8.41 (d, J = 10 Hz, 1H: NH in 1); 8.75 (d, J = 9.5 Hz, 1H: NH in 6); 11.62 (s, 1H: OH).

[0208] EXAMPLE 22: Preparation of the 4ζ-ethylpropylamino-de(4ζ-dimethylamino) pristinamycin I_A

[0209] Strain SP92::pVRC508 is cultured in production medium using 60 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-4-ethylpropylaminophenylalanine dihydrochloride, synthesized as in Example 39-6, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 1.8 litre of must recovered from the 60 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4ζ-ethylpropylamino-de(4ζ-dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 63% 100 mM phosphate buffer, pH 2.9, and 37% of acetonitrile. The fractions containing 4ζ-ethylpropylamino-de(4ζ-dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 16 mg of 4ζ-ethylpropylamino-de(4ζ-dimethylamino) pristinamycin I_A are obtained.

[0210] NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.67 (dd, J = 16 and 6 Hz, 1H: 1H of the CH_2 in 5 β); 0.91 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); 0.95 (t, J = 7.5 Hz, 3H: CH_3 of propyl); 1.14 (t, J = 7 Hz, 3H : CH_3 of the ethyl); 1.15 (mt, 1H: 1H of the CH_2 in 3 β); 1.25 (mt, 1H: 1H of the CH_2 in 3 γ); 1.33 (d, J = 7 Hz, 3H : CH_3 in 1 γ); from 1.45 to 1.65 (mt, 3H: the other H of the CH_2 in 3 γ and CH_2 propyl); 1.63 and 1.75 (2 mts, 1H each: CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.23 and 2.33 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ); 2.37 (d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.80 (dt, J = 13 and 5 Hz, 1H: 1H of the CH_2 in 5 ε); 2.89 (dd, J = 12 and 4 Hz, 1H: 1H of the CH_2 in 4 β); from 3.10 to 3.25 (mt, 3H: 1H of the CH_2 in 3 δ and NCH_2 of the propyl); 3.26 (s, 3H: NCH_3); from 3.25 to 3.40 (mt, 2H: NCH_2 of the ethyl); 3.34 (t, J = 12 Hz, 1H: the other H of the CH_2 in 4 β); 3.54 (mt, 1H: the other H of the CH_2 in 3 δ); 4.57 (dd, J = 7.5 and 6 Hz, 1H: 3 α); 4.76 (broad dd, J = 13 and 7.5 Hz, 1H: the other H of the CH_2 in 5 ε); 4.84 (mt, 1H: 2 α); 4.89 (dd, J = 10 and 1 Hz, 1H : 1 α); 5.21 (dd, J = 12 and 4 Hz, 1H: 4 α); 5.28 (broad d, J = 6 Hz, 1H: 5 α); 5.88 (d, J = 9.5 Hz, 1H: 6 α); 5.91 (mt, 1H: 1 β); 6.48 (d, J = 10 Hz, 1H: NH in 2); 6.60 (d, J = 8 Hz, 2H: aromatic H in 4 ε); 7.03 (d, J = 8 Hz, 2H: aromatic H in 4 δ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.47 (limiting AB, 2H: $1'H_4$ and $1'H_5$); 7.89 (mt, 1H: $1'H_6$); 8.42 (d, J = 10 Hz, 1H : NH in 1); 8.76 (d, J = 9.5 Hz, 1H: NH in 6); 11.62 (s, 1H: OH).

[0211] EXAMPLE 23: Preparation of the 4ζ-trifluoro-methoxy-de(4ζ-dimethylamino) pristinamycin I_A

[0212] Strain SP92::pVRC508 is cultured in production medium using 60 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-4-O-trifluoromethyltyrosine hydrochloride, synthesized as in Example 34-8, in water being added at 16h. At the end of 40h of culture, the 1.8 litres of must recovered from the 60 erlenmeyer flasks

is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times [lacuna] volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in [lacuna] ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4ζ-trifluoromethoxy-de(4ζ-dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected in two portions onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% of acetonitrile. The fractions containing 4ζ-trifluoromethoxy-de(4ζ-dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 46.5 mg of 4ζ-trifluoromethoxy-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

[0213] NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.77 (dd, J = 16 and 5.5 Hz, 1H: 1H of the CH_2 in 5 β); 0.92 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); 1.08 (mt, 1H: 1H of the CH_2 in 3 β); from 1.30 to 1.40 (mt, 1H: 1H of the CH_2 in 3 γ); 1.33 (d, J = 7 Hz, 3H: CH_3 in 1 γ); from 1.55 to 1.70 (mt, 1H: the other H of the CH_2 in 3 γ); 1.65 and 1.76 (2 mts, 1H each: CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.11 and 2.40 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ); 2.54 (d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.88 (dt, J = 13 and 4 Hz, 1H: 1H of the CH_2 in 5 ε); 3.08 (dd, J = 12 and 5 Hz, 1H: 1H of the CH_2 in 4 β); 3.22 (s, 3H: NCH_3); from 3.30 to 3.45 (mt, 1H: 1H of the CH_2 in 3 δ); 3.39 (t, J = 12 Hz, 1H: the

other H of the CH_2 in 4 β); 3.59 (mt, 1H: the other H of the CH_2 in 3 δ); 4.53 (t, J = 7.5 Hz, 1H : 3 α); 4.75 (broad dd, J = 13 and 8 Hz, 1H: the other H of the CH_2 in 5 ε); 4.85 (mt, 1H: 2 α); 4.89 (dd, J = 10 and 1.5 Hz, 1H: 1 α); 5.35 (broad d, J = 5.5 Hz, 1H: 5 α); 5.41 (dd, J = 12 and 5 Hz, 1H: 4 α); 5.92 (d, J = 10 Hz, 1H : 6 α); 5.93 (mt, 1H: 1 β); 6.53 (d, J = 9.5 Hz, 1H: NH in 2); from 7.15 to 7.35 (mt, 5H: aromatic H in 6); 7.16 (d, J = 8 Hz, 2H: aromatic H in 4 ε); 7.26 (d, J = 8 Hz, 2H: aromatic H in 4 δ); 7.37 (dd, J = 8.5 and 4 Hz, 1H: $1'H_5$); 7.42 (dd, J = 8.5 and 1.5 Hz, 1H: $1'H_4$); 7.70 (dd, J = 4 and 1.5 Hz, 1H: $1'H_6$); 8.37 (d, J = 10 Hz, 1H: NH in 1); 8.75 (d, J = 10 Hz, 1H: NH in 6); 11.66 (s, 1H: OH).

[0214] EXAMPLE 24: Preparation of 4ζ-allyloxy-de(4ζ-dimethylamino)pristinamycin I_A

[0215] Strain SP92::pVRC508 is cultured in production medium using 90 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (S)-4-O-allyltyrosine hydrochloride, synthesized as in Example 33, in 0.1N hydrochloric acid being added at 16h. At the end of 40h of culture, the 2.7 litres of must recovered from the 90 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4ζ-allyloxy-de(4ζ-dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column,

which is eluted with a mixture consisting of 52% 100 mM phosphate buffer, pH 2.9, and 48% of acetonitrile. The fractions containing 4ζ-allyloxy-de(4ζ-dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 29 mg of 4ζ-allyloxy-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

[0216] NMR spectrum. ^{1}H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS):0.63 (dd, J = 16 and 6 Hz, 1H: 1H of CH_2 in 5 β); 0.91 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); 1.13 (mt, 1H: 1H of CH_2 in 3 β); 1.29 (mt, 1H: 1H of CH_2 in 3 γ); 1.33 (d, J = 6.5 Hz, 3H: CH_3 in 1 γ); 1.57 (mt, 1H: the other H of the CH_2 in 3 γ); 1.65 and 1.74 (2 mts, 1H each: CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.14 and 2.34 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ); 2.43 (d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.85 (dt, J = 13 and 4 Hz, 1H: 1H of the CH_2 in 5 ε); 2.95 (dd, J = 12 and 4 Hz, 1H: 1H of the CH_2 in 4 β); 3.25 (s, 3H: NCH_3); 3.33 (mt, 1H: 1H of the CH_2 in 3 δ): 3.36 (t, J = 12 Hz, 1H: the other H of the CH_2 in 4 β); 3.56 (mt, 1H: the other H of the CH_2 in 3 δ); 4.51 (limiting AB, 2H: OCH_2 of the allyl); 4.56 (t, J = 7.5 Hz, 1H: 3 α); 4.75 (broad dd, J = 13 and 8 Hz, 1H: the other H of the CH_2 in 5 ε); 4.84 (mt, 1H: 2 α); 4.88 (dd, J = 10 and 1 Hz, 1H: 1 α); 5.27 (dd, J = 12 and 4 Hz, 1H: 4 α); 5.32 (broad d, J = 6 Hz, 1H: 5 α); 5.30 and 5.40 (respectively, mt and dd, J = 17 and 1.5 Hz, 1H each: $=CH_2$ of the allyl); 5.89 (d, J = 9.5 Hz, 1H: 6 α); 5.91 (mt, 1H: 1 β); 6.02 (mt, 1H: CH= of the allyl); 6.50 (d, J = 10 Hz, 1H: NH in 2); 6.85 (d, J = 8 Hz, 2H: aromatic H in 4 ε); 7.12 (d, J = 8 Hz, 2H: aromatic H in 4 δ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.45 (dd, J = 8.5 and 1.5 Hz, 1H: 1'H_4); 7.57 (dd, J = 8.5 and 4 Hz, 1H: 1'H_5); 7.77 (dd, J = 4 and 1.5 Hz, 1H: 1'H_6); 8.41 (d, J = 10 Hz, 1H: NH in 1); 8.74 (d, J = 9.5 Hz, 1H: NH in 6); 11.63 (s, 1H: OH).

[0217] EXAMPLE 25: Preparation of 4ζ-ethoxy-de(4ζ-dimethylamino) pristinamycin I_A

[0218] Strain SP92::pVRC508 is cultured in production medium using 90 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (S)-4-O-ethyltyrosine hydrochloride, synthesized as in Example 33, in 0.1N hydrochloric acid being added at 16h. At the end of 40h of culture, the 2.7 litres of must recovered from the 90 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4ζ-ethoxy-de(4ζ-dimethylamino) pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 52% 100 mM phosphate buffer, pH 2.9, and 48% of acetonitrile. The fractions containing 4ζ-ethoxy-de(4ζ-dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 29 mg of 4ζ-ethoxy-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

[0219] NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.64 (dd, J = 16 and 5.5 Hz, 1H: 1H of the CH_2 in 5 β); 0.90 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); 1.12 (mt, 1H: 1H of the CH_2 in 3 β); 1.25 (mt, 1H: 1H of the CH_2 in 3 γ); 1.33 (d, J = 7 Hz, 3H: CH_3 in 1 γ); 1.42 (t, J = 7

Hz, 3H: CH_3 of the ethyl); 1.57 (mt, 1H: the other H of the CH_2 in 3 γ); 1.63 and 1.74 (2 mts, 1H each: CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.16 and 2.35 (respectively mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ); 2.43 (d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.83 (dt, J = 13 and 4 Hz, 1H: 1H of the CH_2 in 5 ε); 2.93 (dd, J = 12 and 4 Hz, 1H: 1H of the CH_2 in 4 β); from 3.15 to 3.30 (mt, 1H: 1H of the CH_2 in 3 δ); 3.24 (s, 3H: NCH_3); 3.35 (t, J = 12 Hz, 1H: the other H of the CH_2 in 4 β); 3.55 (mt, 1H: the other H of the CH_2 in 3 δ); 3.95 (limiting AB, 2H: OCH_2 of the ethyl); 4.56 (dd, J = 7.5 and 6 Hz, 1H: 3 α); 4.75 (broad dd, J = 13 and 8 Hz, 1H: the other H of the CH_2 in 5 ε); 4.84 (mt, 1H: 2 α); 4.87 (dd, J = 10 and 1 Hz, 1H: 1 α); 5.26 (dd, J = 12 and 4 Hz, 1H: 4 α); 5.32 (broad d, J = 5.5 Hz, 1H: 5 α); 5.88 (d, J = 10 Hz, 1H: 6 α); 5.92 (mt, 1H: 1 β); 6.48 (d, J = 10 Hz, 1H: NH in 2); 6.83 (d, J = 8 Hz, 2H: aromatic H in 4 ε); 7.10 (d, J = 8 Hz, 2H: aromatic H in 4 δ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.44 (dd, J = 8.5 and 1.5 Hz, 1H: $1'H_4$); 7.57 (dd, J = 8.5 and 4.5 Hz, 1H: $1'H_5$); 7.77 (dd, J = 4.5 and 1.5 Hz, 1H: $1'H_6$); 8.38 (d, J = 10 Hz, 1H: NH in 1); 8.75 (d, J = 10 Hz, 1H : NH in 6); 11.60 (s, 1H: OH).

[0220] **EXAMPLE 26: Preparation of 4ζ-(2-chloro-ethoxy)-de(4ζ-dimethylamino) pristinamycin I_A**

[0221] Strain SP92::pVRC508 is cultured in production medium using 60 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (S)-4-O-(2-chloroethyl)tyrosine hydrochloride, synthesized as in Example 42-1, in water being added at 16h. At the end of 40h of culture, the 1.8 litres of must recovered from the 60 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried

over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4ζ-(2-chloroethoxy)-de(4ζ-dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% of acetonitrile. The fractions containing 4ζ-(2-chloroethoxy)-de(4ζ-dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 3.2 mg of 4ζ-(2-chloroethoxy)-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

[0222] NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.66 (dd, J = 16 and 5.5 Hz, 1H: 1H of the CH_2 in 5 β); 0.91 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); 1.13 (mt, 1H: 1H of the CH_2 in 3 β); 1.28 (mt, 1H: 1H of the CH_2 in 3 γ); 1.33 (d, J = 7 Hz, 3H: CH_3 in 1 γ); 1.57 (mt, 1H: the other H of the CH_2 in 3 γ); 1.66 and 1.76 (2 mts, 1H each: CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.16 and 2.37 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ): 2.47 (d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.86 (dt, J = 13 and 4 Hz, 1H: 1H of the CH_2 in 5 ε); 2.95 (dd, J = 12 and 4 Hz, 1H: 1H of the CH_2 in 4 β); 3.23 (s, 3H: NCH_3); 3.32 (mt, 1H: 1H of the CH_2 in 3 δ); 3.37 (t, J = 12 Hz, 1H: the other H of the CH_2 in 4 β); 3.57 (mt, 1H: the other H of the CH_2 in 3 δ); 3.82 (t, J = 6 Hz, 2H: CH_2Cl); 4.19 (limiting AB, 2H: OCH_2 of the ethyl); 4.55 (dd, J = 7.5 and 7 Hz, 1H: 3 α); 4.75 (broad dd, J = 13 and 8 Hz, 1H: the other H of the CH_2 in 5 ε); 4.84 (mt, 1H: 2 α); 4.87 (broad d, J = 10 Hz, 1H: 1 α); 5.28 (dd, J = 12 and 4 Hz, 1H: 4 α); 5.32 (broad d, J = 5.5 Hz, 1H : 5 α); 5.88 (d, J = 10 Hz, 1H: 6α); 5.90

(mt, 1H : 1 β); 6.50 (d, J = 10 Hz, 1H: NH in 2); 6.86 (d, J = 8 Hz, 2H: aromatic H in 4 ε); 7.13 (d, J = 8 Hz, 2H: aromatic H in 4 δ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.45 (limiting AB, 2H: $1'H_4$ and $1'H_5$); 7.75 (dd, J = 4 and 2 Hz, 1H: $1'H_6$); 8.38 (d, J = 10 Hz, 1H: NH in 1); 8.74 (d, J = 10 Hz, 1H: NH in 6); 11.62 (s, 1H: OH).

[0223] EXAMPLE 27: Preparation of 4ζ-acetyl-de 4ζ-dimethylamino) pristinamycin I_A

[0224] Strain SP92::pVRC508 is cultured in production medium using 60 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (S)-4-acetylphenylalanine, synthesized as in Example 33, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 1.8 litres of must recovered from the 60 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4ζ-acetyl)-de(4ζ-dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% of acetonitrile. The fractions containing 4ζ-acetyl-de(4ζ-dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with

water, dried over sodium sulphate and then evaporated. 4.2 mg of 4ζ-acetyl-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

[0225] NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.73 (dd, J = 16 and 6 Hz, 1H: 1H of the CH_2 in 5 β); 0.93 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); 1.12 (mt, 1H: 1H of the CH_2 in 3 β); from 1.25 to 1.45 (mt, 1H: 1H of the CH_2 in 3 γ); 1.33 (d, J = 7 Hz, 3H: CH_3 in 1 γ); 1.62 (mt, 1H: the other H of the CH_2 in 3 γ); from 1.60 to 1.85 (mt, 2H: CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.20 and 2.42 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ); 2.52 (d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.60 (s, 3H: $ArCOCH_3$); 2.88 (dt, J = 13 and 4.5 Hz, 1H: 1H of CH_2 in 5 ε); 3.13 (dd, J = 13.5 and 5.5 Hz, 1H: 1H of the CH_2 in 4 β); 3.21 (s, 3H: NCH_3); from 3.30 to 3.50 (mt, 1H: the other H of the CH_2 in 4 β); from 3.30 to 3.50 and 3.63 (2 mts, 1H each: CH_2 in 3 δ); 4.53 (t, J = 7.5 Hz, 1H: 3 α); 4.75 (broad dd, J = 13 and 8 Hz, 1H: the other H of the CH_2 in 5 ε); 4.84 (mt, 1H: 2 α); 4.88 (dd, J = 10 and 1 Hz, 1H: 1 α); 5.35 (broad d, J = 6 Hz, 1H: 5 α); 5.43 (dd, J = 10. 5 and 4 Hz, 1H: 4 α); 5.90 (d, J = 9.5 Hz, 1H: 6 α); 5.92 (mt, 1H: 1 β); 6.56 (d, J = 9.5 Hz, 1H: NH in 2); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.28 (d, J = 8 Hz, 2H: aromatic H in 4 δ); 7.38 (dd, J = 8.5 and 2 Hz, 1H: $1'H_4$); 7.42 (dd, J = 8.5 and 4.5 Hz, 1H: $1'H_5$); 7.66 (dd, J = 4.5 and 2 Hz, 1H : $1'H_6$); 7.88 (d, J = 8 Hz, 2H: aromatic H in 4 ε); 8.38 (d, J = 10 Hz, 1H: NH in 1); 8.74 (d, J = 9.5 Hz, 1H: NH in 6); 11.65 (s, 1H: OH).

[0226] EXAMPLE 28: Preparation of 4ε-dimethylamino-de(4ζ-dimethylamino) pristinamycin I_A.

[0227] Strain SP92::pVRC508 is cultured in production medium using 60 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-3-dimethylaminophenylalanine dihydrochloride, synthesized as in Example 35-10, in 0.1N sodium

hydroxide solution being added at 16h. At the end of 40h of culture, the 1.8 litres of must recovered from the 60 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4ε-dimethylamino-de(4ζ-dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 3 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 57% 100 mM phosphate buffer, pH 2.9, and 43% of acetonitrile. The fractions containing 4ε-dimethylamino-de(4ζ-dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 1.1 mg of 4ε-dimethylamino-de(4ζ-dimethylamino) pristinamycin I_A are obtained.

[0228] NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.63 (dd, J = 16 and 5 Hz, 1H: 1H of the CH_2 in 5 β); 0.91 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); 1.13 (mt, 1H: 1H of the CH_2 in 3 β); from 1.20 to 1.35 (mt, 1H: 1H of the CH_2 in 3 γ); 1.32 (d, J = 6.5 Hz, 3H: CH_3 in 1 γ); 1.57 (mt, 1H: the other H of the CH_2 in 3 γ); 1.63 and 1.76 (2 mts, 1H each: CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.08 and 2.31 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ): 2.35 (d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.81 (dt, J = 13 and 4 Hz, 1H: 1H of the CH_2 in 5 ε); 2.90 (s, 6H: $N(CH_3)2$); 2.97 (dd, J = 12 and 4 Hz, 1H: 1H of the

CH_2 in 4 β); from 3.20 to 3.30 (mt, 1H: 1H of the CH_2 in 3 δ); 3.28 (s, 3H: NCH_3); 3.37 (t, J = 12 Hz, 1H: the other H of the CH_2 in 4 β); 3.57 (mt, 1H: the other H of the CH_2 in 3 δ); 4.58 (t, J = 7.5 Hz, 1H : 3 α); 4.74 (broad dd, J = 13 and 8 Hz, 1H: the other H of the CH_2 in 5 ε); 4.86 (mt, 1H: 2 α); 4.89 (broad d, J = 10 Hz, 1H: 1 α); 5.27 (dd, J = 12 and 4 Hz, 1H: 4 α); 5.29 (broad d, J = 5 Hz, 1H : 5 α); 5.89 (d, J = 9.5 Hz, 1H: 6 α); 5.90 (mt, 1H: 1 β); 6.50 (d, J = 10 Hz, 1H: NH in 2); from 6.50 to 6.70 (mt, 3H: aromatic Hs in the ortho and in the para positions with respect to the dimethylamino); from 7.15 to 7.35 (mt, 5H: aromatic Hs in 6); 7.20 (t, J = 8 Hz, 1H: aromatic H in the meta position with respect to the dimethylamino); 7.43 (limiting AB, 2H: 1'H_4 and 1'H_5); 7.82 (mt, 1H: 1'H_6); 8.38 (d, J = 10 Hz, 1H: NH in 1); 8.73 (d, J = 9.5 Hz, 1H: NH in 6); 11.61 (s, 1H: OH).

[0229] **EXAMPLE 29: Preparation of 4ϵ-methylthio-de(4ζ-dimethylamino) pristinamycin I_A**

[0230] Strain SP92::pVRC508 is cultured in production medium using 56 erlenmeyer flasks, as described in Example 3, with 1ml of a 20 g/l solution of (R,S)-3-methylthiophenylalanine hydrochloride, synthesized as in Example 34-11, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 1.68 litres of must recovered from the 56 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the novel derivative of pristinamycin I_A

are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 54% of water and 46% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% of acetonitrile. The fractions containing the novel pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 20 mg of 4ε-methylthio-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

[0231] NMR spectrum. ^{1}H (400 MHz, CDCl3, δ in ppm, ref. TMS): 0.56 (dd, J = 16 and 5.5 Hz, 1H: 1H of the CH_2 in 5 β); 0.90 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); 1.13 (mt, 1H: 1H of the CH_2 in 3 β); 1.28 (mt, 1H: 1H of the CH_2 in 3 γ); 1.32 (d, J = 6.5 Hz, 3H: CH_3 in 1 γ); 1.58 (mt, 1H: the other H of the CH_2 in 3 γ); 1.62 and 1.74 (2 mts, 1H each: CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.25 and 2.35 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ); 2.39 (d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.43 (s, 3H: SCH_3); 2.82 (dt, J = 13 and 4 Hz, 1H: 1H of the CH_2 in 5 ε); 2.98 (dd, J = 12 and 4.5 Hz, 1H: 1H of the CH_2 in 4 β); 3.26 (s, 3H: NCH_3); 3.30 (t, J = 12 Hz 1H: 1H of CH_2 in 3 δ); 3.38 (mt, 1H: the other H of the CH_2 in 4 β); 3.57 (mt, 1H: the other H of the CH_2 in 3 δ); 4.56 (t, J = 7.5 Hz, 1H: 3 α); 4.74 (broad dd, J = 13 and 8 Hz, 1H: the other H of the CH_2 in 5 ε); 4.84 (mt, 1H: 2 α); 4.89 (dd, J = 10 and 1 Hz, 1H: 1 α); 5.29 (dd, J = 12 and 4.5 Hz, 1H : 4 α); 5.32 (broad d, J = 5.5 Hz, 1H : 5 α); 5.88 (d, J = 9.5 Hz, 1H: 6 α); 5.90 (mt, 1H: 1 β); 6.51 (d, J = 10 Hz, 1H: NH in 2); 6.99 (broad d, J = 8 Hz, 1H: aromatic H in the para position with respect to the methylthio); 7.10 and 7.15 (respectively, broad s and broad d, J = 8 Hz, 1H each: aromatic Hs in the ortho position with respect to the methylthio); from 7.15 to 7.35 (mt, 6H: aromatic Hs in 6 and aromatic Hs in the meta position with respect to the methylthio); 7.43 (broad d, J = 8 Hz, 1H: 1'H_4); 7.52 (dd, J = 8

and 4 Hz, 1H: 1'H_5); 7.79 (broad d, J = 4 Hz, 1H: 1'H_6); 8.38 (d, J = 10 Hz, 1H: NH in 1); 8.73 (d, J = 9.5 Hz, 1H: NH in 6); 11.62 (s, 1H: OH).

[0232] **EXAMPLE 30: Preparation of 4ε-ethoxy-de(4ζ-dimethylamino) pristinamycin I_A.**

[0233] Strain SP92::pVRC508 is cultured in production medium using 60 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (S)-3-O-ethyltyrosine hydrochloride, synthesized as in Example 37-1, in 0.2N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 1.8 litres of must recovered from the 60 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the novel derivative of pristinamycin I_A are combined and evaporated. 19 mg of dry residue are obtained. The latter is taken up in 3 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% of acetonitrile. The fractions containing the novel pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 15.8 mg of 4ε-O-ethoxy-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

[0234] NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.55 (dd, J = 16 and 5.5 Hz, 1H: 1H of the CH_2 in 5 β); 0.90 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); 1.12 (mt, 1H: 1H of the CH_2 in 3 β); 1.20 (mt, 1H: 1H of the CH_2 in 3 γ); 1.31 (d, J = 6.5 Hz, 3H: CH_3 in 1 γ); 1.49 (t, J = 7 Hz, 3H: CH_3 of the ethyl); 1.54 (mt, 1H: the other H of the CH_2 in 3 γ); 1.63 and 1.73 (2 mts, 1H each: CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.22 and 2.33 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ); 2.46 (d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.83 (dt, J = 13 and 4 Hz, 1H: 1H of the CH_2 in 5 ε); 2.95 (dd, J = 12 and 4 Hz, 1H: 1H of the CH_2 in 4 β); 3.22 (mt, 1H: 1H of the CH_2 in 3 δ); 3.27 (s, 3H: NCH_3); 3.39 (t, J = 12 Hz, 1H: the other H of the CH_2 in 4 β); 3.53 (mt, 1H: the other H of the CH_2 in 3 δ); 3.93 and 4.03 (2 mts, 1H each: OCH_2 of the ethyl); 4.56 (dd, J = 7 and 5.5 Hz, 1H: 3 α); 4.75 (broad dd, J = 13 and 8 Hz, 1H: the other H of the CH_2 in 5 ε); 4.82 (mt, 1H: 2 α); 4.88 (dd, J = 10 and 1 Hz, 1H: 1 α); 5.23 (dd, J = 12 and 4 Hz, 1H: 4 α); 5.23 (broad d, J = 5.5 Hz, 1H: 5 α); 5.87 (d, J = 9.5 Hz, 1H: 6 α); 5.92 (mt, 1H: 1 β); 6.47 (d, J = 10 Hz, 1H: NH in 2); 6.80 (mt, 3H: aromatic H in the ortho and in the para positions with respect to the ethoxy); from 7.10 to 7.35 (mt, 6H: aromatic Hs in 6 and aromatic Hs in the meta position with respect to the ethoxy); 7.43 (dd, J = 8 and 1 Hz, 1H: $1'H_4$); 7.50 (dd, J = 8 and 4 Hz, 1H: $1'H_5$); 7.77 (dd, J = 4 and 1 Hz, 1H: $1'H_6$); 8.38 (d, J = 10 Hz, 1H: NH in 1); 8.70 (d, J = 9.5 Hz, 1H: NH in 6); 11.60 (s, 1H: OH).

[0235] **EXAMPLE 31: Preparation of 4ζ-ethylthio-de (4ζdimethylamino) pristinamycin I_A**

[0236] Strain SP92::pVRC508 is cultured in production medium using 2 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (S)-4-ethylthiophenylalanine hydrochloride, synthesized as in Example 33, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 60 ml of must recovered from the 2 erlenmeyer flasks is

extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4ζ-ethylthio-de(4ζ-dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 52% 100 mM phosphate buffer, pH 2.9, and 48% of acetonitrile. The fractions containing 4ζ-ethylthio-de(4ζ-dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. ? mg of 4ζ-ethylthio-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

[0237] NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm): 0.68 (dd, J = 16 and 6 Hz, 1H: 1H of the CH_2 in 5 β); 0.92 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); from 1.10 to 1.40 (mt, 5H: 1H of the CH_2 in 3 β and 1H of the CH_2 in 3 γ and CH_3 of the ethyl); 1.32 (d, J = 7 Hz, 3H: CH_3 in 1 γ); from 1.45 to 1.85 (mt, 3H: the other H of the CH_2 in 3 γ and CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.18 and 2.37 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH_2 in 5 δ); 2.45 (broad d, J = 16 Hz, 1H: the other H of the CH_2 in 5 β); 2.85 (dt, J = 13 and 4 Hz, 1H: 1H of the CH_2 in 5 ε); 2.90 (mt, 2H: $ArSCH_2$ ethyl); 2.98 (dd, J = 12 and 4 Hz, 1H: 1H of the CH_2 in 4 β); 3.25 (s, 3H: NCH_3); 3.35 (mt, 1H: 1H of the CH_2 in 3 δ); 3.39 (t, J = 12 Hz, 1H: the other H of the CH_2 in 4 β); 3.57 (mt, 1H: the other H of the CH_2 in 3 δ); 4.55 (t, J = 7.5 Hz, 1H: 3

α); 4.75 (broad dd, J = 13 and 7.5 Hz, 1H, : the other H of the CH_2 in 5 ε); 4.85 (mt, 1H: 2 α); 4.89 (dd, J = 10 and 1 Hz, 1H: 1 α); from 5.25 to 5.40 (mt, 2H: 5 α and 4 α); 5.88 (d, J = 9.5 Hz, 1H: 6 α); 5.91 (mt, 1H: 1 β); 6.55 (d, J = 9.5 Hz, 1H: NH in 2); 7.10 (d, J = 8 Hz, 2H: aromatic Hs in 4 δ); from 7.10 to 7.35 (mt, 7H: aromatic Hs in 6 and 4 ε); 7.44 (limiting AB, 2H: 1'H_4 and 1'H5); 7.74 (mt, 1H: 1'H_6); 8.38 (d, J = 10 Hz, 1H: NH in 1); 8.75 (d, J = 9.5 Hz, 1H: NH in 6); 11.62 (s, 1H: OH).

[0238] **EXAMPLE 32: Preparation of 4ζ-ethyl-de(4ζ-dimethylamino) pristinamycin I_A**

[0239] Strain SP92::pVRC508 is cultured in production medium using 2 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-4-ethylphenylalanine, synthesized as in Example 33, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 60 ml of must recovered from the 2 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times [lacuna] volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4ζ-ethyl-de(4ζ-dimethylamino) pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 52% of water and 48% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 52% 100 mM phosphate buffer, pH 2.9, and 48% of acetonitrile. The fractions containing 4ζ-ethyl-de(4ζ-dimethylamino)pristinamycin I_A are

combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 0.50 mg of 4ζ-ethyl-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

[0240] NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm, ref. TMS): 0.42 (dd, J = 16 and 5.5 Hz, 1H: 1H of the CH_2 in 5 β); 0.92 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); from 1.10 to 1.40 (mt, 2H: 1H of the CH_2 in 3 β and 1H of the CH_2 in 3 γ); 1.23 (t, J = 7.5 Hz, 3H: CH_3 of the ethyl); 1.35 (d, J = 7 Hz, 3H: CH_3 in 1 γ); from 1.45 to 1.85 (mt, 3H: the other H of the CH_2 in 3 γ and CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.15 and from 2.25 to 2.40 (2 mts, 1H each: CH_2 in 5 δ); from 2.25 to 2.40 (mt, 1H: the other H of the CH_2 in 5 β); 2.60 (q, J = 7.5 Hz, 2H: $ArCH_2$ of the ethyl); 2.83 (dt, J = 13 and 4 Hz, 1H: 1H of the CH_2 in 5 ε); 2.98 (dd, J = 12 and 4 Hz, 1H: 1H of the CH_2 in 4 β); from 3.25 to 3.35 (mt, 1H: 1H of the CH_2 in 3 δ); 3.27 (s, 3H: NCH_3); 3.39 (t, J = 12 Hz, 1H: the other H of the CH_2 in 4 β); 3.59 (mt, 1H: the other H of the CH_2 in 3 δ); 4.58 (dd, J = 7 and 6.5 Hz, 1H: 3 α); 4.75 (broad dd, J = 13 and 8 Hz, 1H: the other H of the CH_2 in 5 ε); 4.87 (mt, 1H: 2 α); 4.89 (dd, J = 10 and 1 Hz, 1H: 1 α); 5.24 (broad d, J = 5.5 Hz, 1H: 5 α); 5.29 (dd, J = 12 and 4 Hz, 1H: 4 α); 5.88 (d, J = 10 Hz, 1H: 6 α); 5.92 (mt, 1H: 1 β); 6.73 (d, J = 10 Hz, 1H: NH in 2); from 7.10 to 7.35 (mt, 9H: aromatic Hs in 6 - 4 ε and 4 δ); 7.44 (dd, J = 8.5 and 1.5 Hz, 1H: $1'H_4$); 7.50 (dd, J = 8.5 and 4.5 Hz, 1H: $1'H_5$); 7.80 (dd, J = 4.5 and 1.5 Hz, 1H: 1'H6); 8.38 (d, J = 10 Hz, 1H: NH in 1); 8.75 (d, J = 10 Hz, 1H: NH in 6); 11.66 (s, 1H: OH).

[0241] Using the same fractions derived from the silica column described above, which fractions also contain the novel pristinamycin I_H derivative, 0.3 mg of ζ-ethyl-de(4ζ-dimethylamino) pristinamycin I_H is isolated by carrying out semi-preparative column chromatography as described above.

[0242] NMR spectrum. ^1H (400 MHz, $CDCl_3$, δ in ppm): 0.04 (mt 1H: 1H of the CH_2 in 5 β); 0.92 (t, J = 7.5 Hz, 3H: CH_3 in 2 γ); from 1.10 to 1.40 (mt, 2H: 1H of the CH_2 in 5 δ and 1H of the CH_2 in 5 γ); 1.18 (t, J = 7.5 Hz, 3H: CH_3 of the ethyl); 1.30 (d, J = 6.5 Hz, 3H: CH_3 in 1 γ); from 1.45 to 1.85 (mt, 7H: the other H of the CH_2 in 5 γ - the other H of the CH_2 in 5 δ - 1H of the CH_2 in 3 β - CH_2 in 3 γ and CH_2 in 2 β); 1.81 (broad d, J = 13 Hz, 1H: the other H of the CH_2 in 5 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.40 (dt, J = 13 and 4 Hz, 1H: 1H of the CH_2 in 5 ε); 2.65 (q, J = 7.5 Hz, 2H: $ArCH_2$ of the ethyl); 2.97 and 3.09 (respectively, dd and t, J = 12 and 5 Hz and J = 12 Hz, 1H each: CH_2 in 4 β); 3.50 and 3.60 (2 mts, 1H each: CH_2 in 3 δ); 4.13 (dd, J = 8 and 5 Hz, 1H: 3 α); 4.49 (broad d, J = 13 Hz, 1H: the other H of the CH_2 in 5 ε); 4.70 (mt, 2H: 5 α and 4 α); 4.77 (mt, 1H: 2 α); 4.83 (dd, J = 10 and 1 Hz, 1H: 1 α); 5.50 (d, J = 7 Hz, 1H: 6 α); 5.74 (mt, 1H: 1 β); 6.09 (d, J = 4 Hz, 1H: NH in 4); 6.72 (unres. comp., 1H: NH in 2); 7.07 (d, J= 8 Hz, 2H: aromatic Hs in 4 ε); 7.15 (d, J = 8 Hz, 2H: aromatic Hs in 4 δ); from 7.15 to 7.35 (mt, 5H: aromatic Hs in 6); 7.40 (dd, J = 8 and 1 Hz, 1H: $1'H_4$); 7.45 (dd, J = 8 and 4 Hz, 1H: $1'H_5$); 7.92 (dd, J = 4 and 1 Hz, 1H: 1'H6); 8.40 (unres. comp., 1H: NH in 6); 8.50 (d, J = 10 Hz, 1H: NH in 1); 11.72 (s, 1H: OH).

[0243] EXAMPLE 33: Preparation of derivatives of phenylalanine and of phenylpyruvic acid which have already been described.

[0244] Phenylalanine, and its derivatives 4-methoxyphenylalanine, 4-bromophenylalanine, 4-chlorophenylalanine, 4-iodophenylalanine, 4-trifluoromethylphenylalanine, 4-aminophenylalanine and 3-methoxyphenylalanine, which are employed in this work, are commercially available.

[0245] The following derivatives of phenylalanine can be prepared in accordance with methods described in the literature.

[0246] **(RS)-4-dimethylaminophenylalanine**

[0247] D.F. Elliott, A.T. Fuller, C.R. Harrington, J. Chem. Soc., 1948, 85-89.

[0248] **(RS)-4-diethylaminophenylalanine**

[0249] Moldaver B.L., Pushkareva Z.V., Zhur. Obshchei Khim., 31, 1560-1569 (1961); C.A. 1961, 22226f.; J.A. Stock, J. Chem. Soc, 1959, 90-97

[0250] (RS)-4-ethylaminophenylalanine

[0251] F. Bergel, J.A. Stock, J. Chem. Soc, 1959, 90-97.

[0252] **(RS)-4-phenylphenylalanine**

[0253] J.V. Braun, J. Nelles, Berichte, 66B, 1933, 1464-1470.

[0254] **(RS)-4-methylphenylalanine**

[0255] R.R., Herr, T. Enjoki, J.P. Dailey, J. Am. Chem. Soc, 1957, 79, 4229-4231.

[0256] **(RS)-4-methylthiophenylalanine and (R,S)-4-ethylthiophenylalanine**

[0257] R.L. Colescott, R.R. Herr, J.P. Dailey J. Am. Chem. Soc, 1957, 79, 4232-4235.

[0258] **(RS)-4-methoxycarbonylphenylalanine**

[0259] H. Cleland, J. Org. Chem., 1969, 34, 747.

[0260] **(RS)-2,4-dimethylphenylalanine**

[0261] R.R., Herr, T. Enjoki, J.P. Dailey, J. Am. Chem. Soc, 1957, 79, 4229-4231.

[0262] **(RS)-3,4-dimethylphenylalanine**

[0263] R.R., Herr, T. Enjoki, J.P. Dailey, J. Am. Chem. Soc, 1957, 79, 4229-4231.

[0264] **(RS)-3-trifluoromethylphenylalanine hydrochloride**

[0265] R. Filler and H. Novar. J. Org. Chem, 1960, 25, 733-736.

[0266] **(S)-4-aminomethylphenylalanine**

[0267] G.E. Stokker, W.F. Hoffman and C.F. Homnick, J. Org. Chem., 1993, 58, 5015-5017.

[0268] **(R,S)-3-methylphenylalanine**

[0269] J.H. Burckhalter, V.C. Stephens, J.A.C.S. 1951, 73, 56-58.

[0270] **(R,S)-4-acetylphenylalanine**

[0271] J.I. Degaw et al., J. Med.Chem., 1969, 11, 225-227

[0272] **(S)-4-O-allyltyrosine**

[0273] A. Loffet, H. Zang, Int. J. Pept. Protein. Res., 1993, 42, 346

[0274] **(S)-4-O-ethyltyrosine**

[0275] Y. Sasaki et al., Chem. Pharm. Bull., 1982, 30, 4435

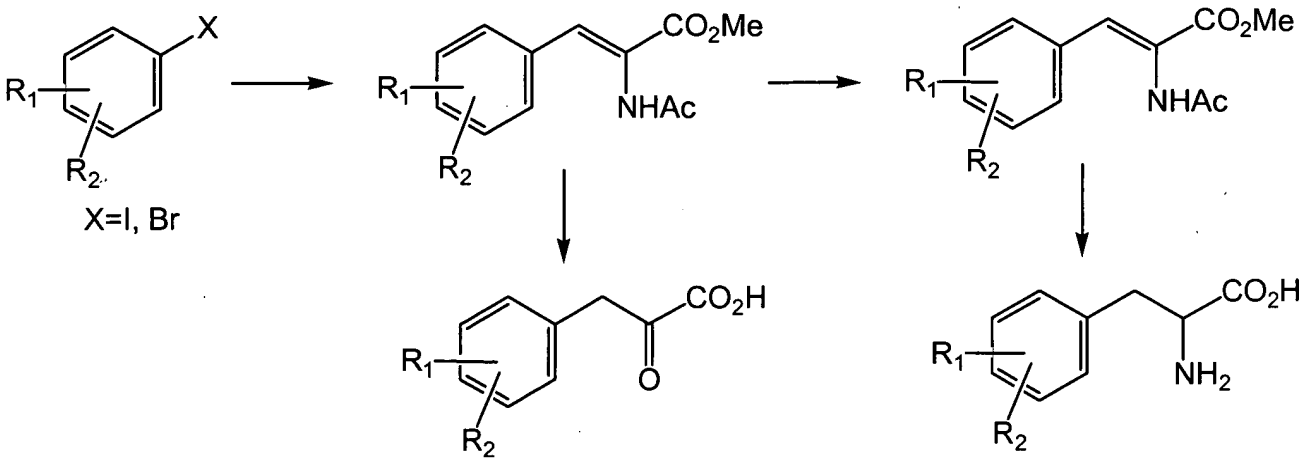
[0276] **(RS)-4-ethylphenylalanine**

[0277] A. Zhuze et al., Coll., Czech. Chem. Comm., 1965, 62, 2648

[0278] **4-tert-butylphenylpyruvic acid** can be prepared in accordance with R. Breslow, J.W. Canary, M. Varney, S.T. Waddell and D. Yang, J. Am. Chem. Soc., 1990, 112, 5212-5219.

[0279] The other derivatives of phenylalanine were prepared in accordance with Examples 34 to 42 which are given below. In these examples, flash chromatography was carried out under a mean nitrogen pressure of 50 kPa using a silica of granule size 40-53 μm, in accordance with Still et al., J. Org. Chem., 43, 2923, (1978).

[0280] EXAMPLE 34: Preparation of derivatives of phenylalanine and of a derivative of phenylpyruvic acid using method A.



[0281] 34-1 (RS)-4-methylaminophenylalanine, dihydrochloride

[0282] 37 ml of 12 N hydrochloric acid are added to 3.70 g of methyl N-acetyl-4-methylaminophenylalaninate, and the mixture is heated to reflux, while stirring, for 8 h. After one night at room temperature, the reaction medium is concentrated to dryness under reduced pressure (50 kPa), and the residue is taken up in a mixture of 50 ml of toluene and 50 ml of ethanol, and this mixture is concentrated once again. After drying in a desiccator under reduced pressure (2.6 kPa), 4.18 g (100%) of (RS)-4-methylaminophenylalanine dihydrochloride are obtained in the form of a hygroscopic light beige solid which melts at 158°C.

[0283] 34-2: Methyl (RS)-N-acetyl-4-methylaminophenylalaninate

[0284] 0.4 g of 10% palladium on charcoal, and then 50 ml of absolute ethanol, are added to 4 g of methyl 4-methylamino-2-acetamidocinnamate which is placed under a nitrogen atmosphere in an autoclave. The mixture is placed under a pressure of 5.5 bar of hydrogen and heated at 50°C for 15 h with stirring. After stabilizing the temperature at 26°C, and returning the pressure to atmospheric, the medium is filtered through Clarcel®, washed with ethanol and then concentrated to dryness under reduced pressure (2.6 kPa). This results in 3.73 g of methyl N-acetyl-4-methylaminophenylalaninate in the form of white crystals which melt at 118°C.

[0285] 34-3: Methyl 4-methylamino-2-acetamidocinnamate

[0286] 5.75 g of methyl 2-acetamidoacrylate, 0.185 g of palladium acetate, 8.1 g of tetrabutylammonium chloride and 6.03 g of sodium hydrogen carbonate are added to a 3-necked flask which is placed under nitrogen, and then 6.5 g of 4-iodo-N-methylalanine, in solution in 200 ml of DMF, are added to this mixture. The mixture is heated at 82°C for 16 h 30 min and then, after having been cooled down, is poured into 1000 ml of distilled water. The medium is extracted with 250 ml of CH_2Cl_2 and the organic phase is separated off; the aqueous phase is then washed twice with 250 ml of CH_2Cl_2. The organic phases are combined, dried over sodium sulphate, filtered and concentrated under reduced pressure (50 kPa) at 70°C to yield a brown oil which is purified by flash chromatography (eluent, AcOEt/cyclohexane and then pure AcOEt).

[0287] In this way, 4 g of methyl 4-methylamino-2-acetamidocinnamate is obtained in the form of a yellow solid (Merck Silica 5719, Rf = 0.48), which is employed in this form.

[0288] N-Methyl-p-iodoanaline can be prepared in accordance with: S. Krishnamurthy, Tetrahedron Letters, 33, 3315-3318, 1982.

[0289] 34-4: 4-methylaminophenylpyruvic acid

[0290] 2.4 g of methyl 4-methylamino-2-acetamidocinnamate and 32 ml of 12 N hydrochloric acid are placed in a round-bottomed flask. The mixture is heated to reflux for 3 h and then cooled down and washed twice with 20 ml of diethyl ether. The aqueous phase is cooled down to -10°C and the precipitate which is obtained is filtered and then rinsed with a minimum of cold hydrochloric acid. The solid which is obtained is dried in a desiccator under reduced pressure in order to yield 1.1 g of 4-methylaminophenylpyruvic acid in the form of a light beige solid which melts at 210°C.

[0291] 34-5: (R,S)-3-Fluoro-4-methylphenylalanine hydrochloride

[0292] 0.6 g of (R,S)-3-fluoro-4-methylphenylalanine hydrochloride is obtained in the form of white crystals which melt at a temperature greater than 260°C by proceeding as in Example 34-1 but using 1.6 g of methyl N-acetyl(3-fluoro-4-methyl)phenylalaninate.

[0293] 34-6: Methyl (R,S)-N-acetyl-(3-fluoro-4-methyl)phenylalaninate

[0294] 1.6 g of methyl N-acetyl-(3-fluoro-4-methyl)phenylalaninate are obtained in the form of a colourless oil (Merck Silica 5719, R_f =0.46; eluent CH_2Cl_2/AcOEt 50/50), by proceeding as in Example 34-2 but using 1.9 g of methyl (4-methyl-3-fluoro)-2-acetamidocinnamate and 0.2 g of 10% palladium on charcoal in 230 ml of ethanol.

[0295] 34-7: Methyl (3-fluoro-4-methyl)-2-acetamidocinnamate

[0296] 2.6 g of methyl (3-fluoro-4-methyl)-2-acetamidocinnamate are obtained in the form of a white solid which melts at 163°C by proceeding as in Example 34-3 but using 3.6 g of methyl 2-acetamidoacrylate, 0.12 g of palladium acetate, 5.2 g of tetrabutylammonium chloride, 3.8 g of sodium hydrogen carbonate and 4 g of 2-fluoro-4-bromotoluene in solution in 120 ml of anhydrous DMF.

[0297] 34-8: (R,S)-4-Trifluoromethoxyphenylalanine hydrochloride or (R,S)-O-trifluoromethyltyrosine hydrochloride

[0298] 1.5 g of (R,S)-4-trifluoromethoxyphenylalanine hydrochloride are obtained in the form of white crystals which melt at 260°C by proceeding as in Example 34-1 but using 3 g of methyl N-acetyl-(4-trifluoromethoxy)phenylalaninate and 30 ml of 12 N hydrochloric acid.

[0299] 34-9: Methyl (R,S)-N-acetyl-(4-trifluoromethoxy)phenylalaninate

[0300] 3 g of methyl N-acetyl-(4-trifluoroethoxy)-phenylalaninate are obtained in the form of a white solid which melts at 80°C by proceeding as in Example 34-2 but using 3.1 g of

methyl (4-trifluoromethoxy)-2-acetamidocinnamate and 0.3 g of 10% palladium on charcoal in 50 ml of ethanol.

[0301] 34-10: Methyl 4-trifluoromethoxy-2-acetamidocinnamate

[0302] 3.1 g of methyl (4-trifluoromethoxy)-2-acetamidocinnamate are obtained in the form of a white solid which melts at 135°C by proceeding as in Example 34-3 but using 4.3 g of methyl 2-acetamido acrylate, 0.14 g of palladium acetate, 6.1 g of tetrabutyl-mmonium chloride, 4.6 g of sodium hydrogen carbonate and 5 g of 4-trifluoromethoxybromobenzene in solution in 150 ml of anhydrous DMF.

[0303] 34-11: (R,S)-3-Methylthiophenylalanine hydrochloride

[0304] 1.38 g of (R,S)-3-methylthiophenylalanine hydrochloride are obtained in the form of white crystals which melt at 190°C by proceeding as in Example 34-1 but using 3.3 g of methyl N-acetyl-3-methylthiophenylalaninate and 40 ml of 12 N hydrochloric acid.

[0305] 34-12: Methyl (RS)-N-acetyl-3-methylthiophenylalaninate

[0306] 3.72 g of methyl 3-methylthio-2-acetamidocinnamate, dissolved in 100 ml of methanol, and 30 ml of tetrahydrofuran are placed in a round-bottomed flask, and 1.4 g of magnesium are then added. After reacting for 20 min, the mixture is cooled in an ice bath and a further 1.4 g of magnesium are then added. The mixture is stirred at room temperature for 18 h and then poured into 1.4 l of distilled water and 300 ml of CH_2Cl_2; this mixture is then filtered through Clarcel®. The aqueous phase is adjusted to pH 6 by adding 12 N hydrochloric acid and then separated off and washed with 100 ml of CH_2Cl_2. The organic phases are collected, dried over magnesium sulphate, filtered and then concentrated to dryness under reduced pressure in order to yield 3.42 g of methyl N-acetyl-3-methylthiophenylalaninate in the form of a colourless oil (Merck Silica 5719, Rf=0.5; AcOEt).

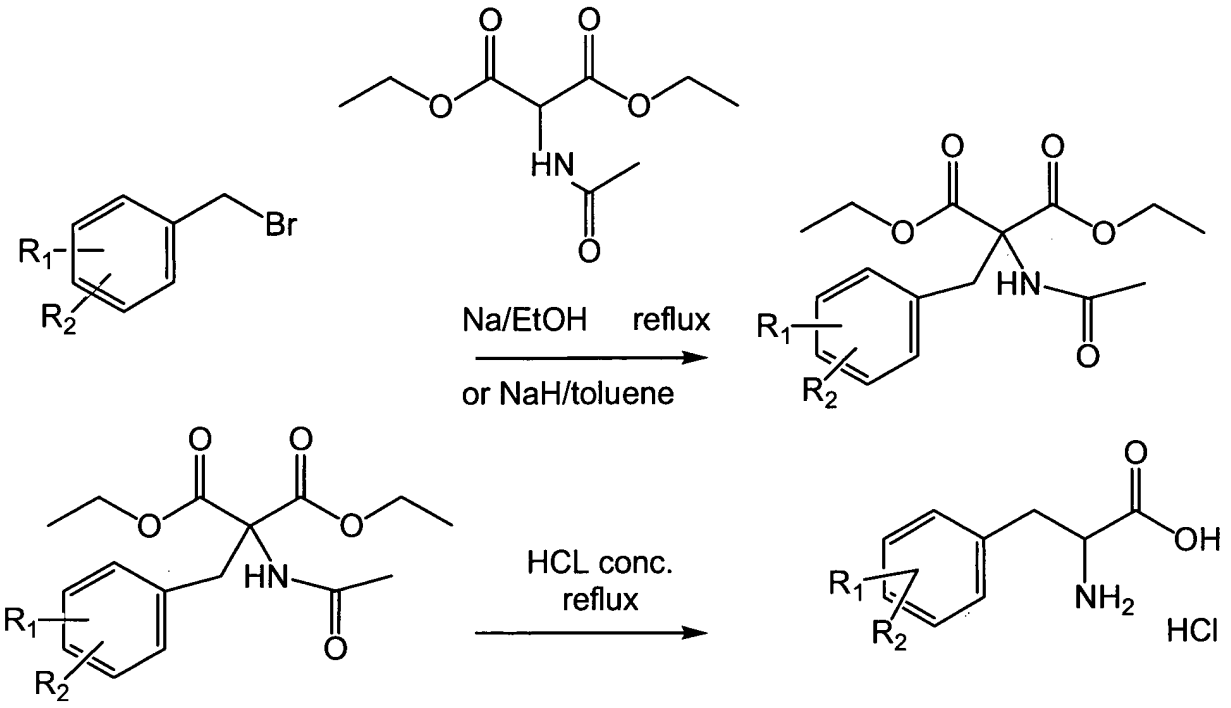
[0307] 34-13: Methyl 3-methylthio-2-acetamidocinnamate

[0308] 4.8 g of methyl (3-methylthio)-2-acetamidocinnamate are obtained in the form of a white solid which melts at 139°C by proceeding as in Example 34-3 but using 5.6 g of methyl 2-acetamidoacrylate, 0.18 g of palladium acetate, 8.2 g of tetrabutylammonium chloride, 5.86 g of sodium hydrogen carbonate and 6.5 g of 3-iodo-1-methylthiobenzene dissolved in 160 ml of anhydrous DMF.

[0309] 34-14: 3-Iodomethylthiobenzene

[0310] 20 ml of distilled water and 20 ml of 12 N hydrochloric acid are placed, with stirring, in a three-necked flask, and 10 ml of 3-methylthioaniline are then added using a dropping funnel. The mixture is warmed to ensure dissolution and is then cooled down to 5°C. 5.86 g of sodium nitrite dissolved in 15 ml of water are subsequently added slowly, using a dropping funnel, while maintaining the temperature between 5 and 8°C. 20 min after having completed the addition, 13.57 g of potassium iodide dissolved in 15 ml of water are added over a period of 10 min and the mixture is then stirred at room temperature for 15 h. The oil which forms is separated from the aqueous phase by decantation, and an aqueous solution of sodium thiosulphate is then added to it. The aqueous phase is decanted and the product is extracted with 100 ml of dichloromethane. The organic phase is washed with 100 ml of water, and the aqueous phase is adjusted to pH 9 with concentrated sodium hydroxide solution, and then separated off. The organic phase is washed with 2 times 100 ml of water, separated off, dried over magnesium sulphate, filtered and then concentrated to dryness under reduced pressure (50 kPa) at 40°C. The resulting product is purified by flash chromatography (eluent, cyclohexane) in order to yield 13 g of 3-iodo-1-methylthiobenzene in the form of a yellow liquid (Merck Silica 5719, R_f=0.8/cyclohexane).

[0311] EXAMPLE 35: Preparation of derivatives of phenylalanine using method B.



[0312] 35-1: (RS)-4-tert-butylphenylalanine

[0313] 25 g of diethyl 4-(tert-butyl)benzyl acetamidomalonate and 250 ml of 37% hydrochloric acid are added to a three-necked flask which is surmounted by a condenser. The mixture is stirred and heated to reflux until there is no further evolution of gas. After the reaction medium has been cooled down, the precipitate which is obtained is filtered and then recrystallized in acetonitrile to yield 25.6 g of (R,S)-4-tert-butylphenylalanine hydrochloride in the form of a white solid which melts at 234°C.

[0314] 35-2: Diethyl 4-(tert-butyl)benzylacetamidomalonate

[0315] 25 g of 4-(tert-butyl)benzyl bromide, 50 ml of anhydrous toluene and 3.1 g of sodium hydride in 80% suspension in oil are added to a three-necked flask which is surmounted by a condenser, followed by 21.8 g of diethyl acetamidomalonate. The mixture is heated at 110°C for 17 h. After it has been cooled down, 15 ml of absolute ethanol, then 15 ml of 50%

ethanol and then 50 ml of water are added slowly to it using a dropping funnel. The organic phase is decanted and the aqueous phase is washed with 3 times 50 ml of diethyl ether. The organic phases are combined, washed with water and then dried over sodium sulphate. Following filtration and concentration under reduced pressure, the product is crystallized in petroleum ether in order to yield 25 g of diethyl 4-(tert-butyl)benzylacetamidomalonate in the form of a white solid which melts at 80°C.

[0316] 35-3: (R,S)-3-Methylaminophenylalanine dihydrochloride

[0317] 1.03 g of a yellow-beige solid are obtained by proceeding as in Example 35-1 but using 1.17 g of diethyl 3-methylaminobenzylacetamidomalonate and 20 ml of 12 N hydrochloric acid. This yellow-beige solid is dissolved in 20 ml of absolute ethanol, and 0.4 g of animal charcoal is added to this solution. The solution is filtered through Clarcel and then filtered and concentrated under reduced pressure (50 kPa). The same procedure is repeated starting with 1 g of animal charcoal, and the solid which is obtained is triturated in 20 ml of ether. Following filtration and drying under reduced pressure (2.7 kPa) at 50°C, 0.65 g of (R,S)-3-methylaminophenylalanine dihydrochloride is obtained in the form of a white powder which melts at a temperature approaching 135°C (decomposition).

[0318] 35-4: Diethyl 3-methylamino benzyl acetamido malonate

[0319] 3.11 ml of acetic anhydride are placed in a three-necked flask which is maintained under a nitrogen atmosphere. 1.51 ml of formic acid are subsequently added within 3 min at 0°C, and the mixture is then heated at 50°C for 2 hours. The mixture is allowed to return to room temperature, while shaking for 3 h 20 min, and 4 ml of anhydrous THF are added under nitrogen; the mixture is then cooled to -20°C. A solution of 4 g of diethyl 3-aminobenzylacetamidomalonate in a mixture of 15 ml of anhydrous THF and 15 ml of

anhydrous dichloromethane is added within 10 min. Stirring is continued for 1 h 10 min at -20°C and then for 16 h at 20°C. The reaction mixture is concentrated to dryness under reduced pressure (50 kPa) at 30°C and then co-evaporated with 30 ml of anhydrous toluene in order to yield a white solid, which is dissolved in a mixture of 10 ml of anhydrous THF and 20 ml of anhydrous 1,2-dichloroethane, which solution is then placed in a three-necked flask under nitrogen.

[0320] The medium is cooled down to -5°C, and 1.55 ml of borane-dimethyl sulphide complex (2M solution in THF) are then added within 10 min. The mixture is allowed to return to room temperature, and the solution is heated to reflux for 3 h and then stirred at room temperature for 15 h. The reaction medium is cooled to 0°C, and 10 ml of MeOH are then added within 25 min. The mixture is stirred for 45 min at 0°C and then for 30 min at room temperature. It is then cooled to 0°C and HCl gas is bubbled in until a pH of 2 is reached. The mixture is heated at reflux for 1 h and is then concentrated to dryness under reduced pressure at 30°C in order to yield 5 g of a product which is taken up in 30 ml of an aqueous solution of $NaHCO_3$ and 30 ml of CH_2Cl_2. The organic phase is decanted and the aqueous phase is washed with 20 ml of water. The organic phases are pooled, dried over magnesium sulphate, filtered and then concentrated to dryness under reduced pressure (2.6 kPa) in order to yield 3.43 g of a yellow oil, which is purified by flash chromatography (eluent, AcOEt/cyclohexane 50/50). After drying under reduced pressure (2.7 kPa) at 20°C, 1.18 g of diethyl 3-methylaminobenzylacetamidomalonate are thus obtained in the form of a light beige solid which melts at 122°C.

[0321] 35-5: Diethyl 3-aminobenzylacetamidomalonate

[0322] Diethyl 3-aminobenzylacetamidomalonate can be prepared as described in:

[0323] T.S. Osdene, D.N. Ward, W.H. Chapman and H. Rakoff, J. Am. Chem. Soc., 81, 1959, 3100-3102.

[0324] 35-6: (R,S)-3-Ethylaminophenylalaninedihydrochloride

[0325] 1.7 g of (R,S)-3-ethylaminophenylalanine dihydrochloride are obtained in the form of a hygroscopic light beige solid, which contains 10 molar % of (R,S)-3-diethylaminophenylalanine dihydrochloride, by proceeding as in Example 34-1 but using 2 g of ethyl (R,S)-N-acetyl-3-ethylamino-phenyl-alaninate and 30 ml of 12N hydrochloric acid.

[0326] 35-7: (R,S)-N-acetyl-3-ethylaminophenyl-alaninate

[0327] 3 g of ethyl (R,S)-N-acetyl-3-aminophenyl-alaninate, 40 ml of ethanol and 14 g of Raney nickel, which has previously been washed with distilled water and ethanol, are placed in a round-bottomed flask under a nitrogen atmosphere. The mixture is heated to reflux for 19 h, cooled down, filtered through Clarcel®, and then concentrated to dryness under reduced pressure (50 kPa) in order to yield 3.07 g of a colourless oil, which is purified by flash chromatography (eluent, AcOet) in order to yield 2.1 g of ethyl (R,S)-N-acetyl-3-ethylaminophenylalaninate in the form of a colourless oil (Merck Silica 5719, R_f=0.6: AcOEt) which contains 10% ethyl (R,S)-N-acetyl-3-diethylaminophenylalaninate.

[0328] 35-8: Ethyl (R,S)-N-acetyl-3-aminophenylalaninate

[0329] 25 g of a mixture of ethyl (R,S)-N-acetyl-3-nitrophenylalaninate (75 mol %/mol) and diethyl 3-nitrobenzylacetamidomalonate (25 mol %/mol) are placed under nitrogen in an autoclave. 2.5 g of 10% palladium on charcoal and then 200 ml of dichloromethane are added. The mixture is placed under a hydrogen pressure of 9 bar and then stirred at 18°C for 4 h. After returning the pressure to atmospheric, the reaction medium is filtered through Clarcel®, washed with dichloromethane and then concentrated to dryness under reduced pressure (50 kPa) in order

to yield a solid, which is recrystallized in 450 ml of distilled water under reflux and in the presence of 4 g of 3S animal charcoal. Following hot filtration through Clarcel®, the mixture is left to crystallize at 4°C, with the crystals being filtered and then dried in order to yield 9.9 g of ethyl (R,S)-N-acetyl-3-aminophenylalaninate in the form of a light beige solid which melts at 106°C and which contains 5% of diethyl 3-aminobenzylacetamidomalonate.

[0330] 35-9: Ethyl (R,S)-N-acetyl-3-nitrophenyl-alaninate and diethyl 3-nitrobenzylacetamidomalonate

[0331] 600 ml of absolute ethanol and then 7.9 g of sodium are placed, under a nitrogen atmosphere, in a three-necked flask which is surmounted by a condenser. Once dissolution is complete, 74.5 g of diethyl acetamidomalonate and then 60 g of 4-nitrobenzyl chloride in 200 ml of anhydrous ethanol are added. The mixture is heated to reflux for 16 h 30 min. After cooling, the reaction medium is concentrated under reduced pressure (50 kPa) and then taken up in a mixture of 500 ml of CH_2Cl_2 and 500 ml of water. The pH is adjusted to 7 by adding 0.5N sulphuric acid, and the organic phase is then separated off and the aqueous phase is washed with 2 times 200 ml of CH_2Cl_2. The organic phases are pooled, washed with 200 ml of water saturated with sodium bicarbonate, separated off and then dried over magnesium sulphate. Following filtration and concentration under reduced pressure (50 kPa), the product is recrystallized in 600 ml of ethanol at reflux in order to yield, after crystallizing at ambient temperature, filtering and drying, 70.4 g of diethyl 3-nitrobenzylacetamido- malonate in the form of white crystals which melt at 156°C. The mother liquors are concentrated and then purified by flash chromatography (eluent, AcOEt) in order to yield 25.6 g of a mixture of ethyl N-acetyl-3-nitrophenylalaninate (75 mol %/mol) and diethyl 3-nitrobenzylacetamidomalonate (25 mol %/mol) in the form of a light beige solid, which is used in this form in the following step.

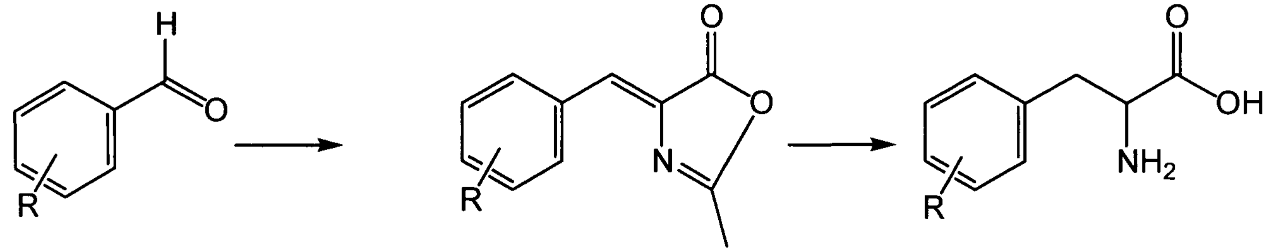
[0332] 35-10: (RS,)-3-Dimethylaminophenylalanine dihydrochloride

[0333] A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 0.72 g of ethyl (RS)-N-acetyl-3-dimethylaminophenylalaninate and 8.6 ml of 10N hydrochloric acid; the solid is subsequently triturated in 50 ml of acetone, filtered and then dried under reduced pressure (2.7 kPa) at 40°C. 0.68 g (93%) of (RS)-3-dimethylaminophenylalanine dihydrochloride is obtained in the form of a white solid which melts in the region of 120°C (decomposition).

[0334] 35-11: Ethyl (RS)-N-acetyl-3-dimethylaminophenylalaninate

[0335] 4 g of ethyl (RS)-N-acetyl-3-aminophenylalaninate, prepared as described in Example 35-8, in 15 ml of DMF are placed in a three-necked flask under a nitrogen atmosphere, and 5.5 ml of triethylamine, and then 2.5 ml of methyl iodide and 4 ml of dichloromethane, are added while maintaining the temperature in the region of 30°C using an icebath. The mixture is then warmed at 35°C for 18h. 1 ml of methyl iodide dissolved in 1 ml of DMF is then added slowly while maintaining the temperature in the region of 30°C; 2.2 ml of triethylamine are then added and the mixture is subsequently warmed for a further 5h at 35°C. The mixture is brought to room temperature and then extracted with 100 ml of ethyl acetate and 150 ml of distilled water. The aqueous phase is separated off after settling and then rewashed with 2 times 70 ml of ethyl acetate. The organic phases are combined, washed with 2 times 80 ml of distilled water and then with 50 ml of distilled water which is saturated with NaCl. The organic phase is separated off after settling, dried over magnesium sulphate, filtered and then concentrated to dryness under reduced pressure in order to yield 2.4 g of a product which is purified by flash chromatography (dichloromethane, MeOH 90/10). 0.72 g (16%) of ethyl (RS)-3-N-acetyl-3-dimethylamino phenylalaninate is thus obtained in the form of yellow crystals.

[0336] EXAMPLE 36: Preparation of derivatives of phenylalanine using method C.



[0337] 36-1: (R,S)-4-Isopropylphenylalanine

[0338] 7 g of red phosphorus and 8 g of 4-(isopropylbenzylidene)-2-methyl-5-oxazolone, in 45 ml of acetic anhydride, are placed in a three-necked flask, and then 35 ml of 57% hydriodic acid are added slowly, with stirring, using a dropping funnel. Once the addition is complete, the mixture is heated to reflux for 3 h 30 min and then left at room temperature for 3 days. The reaction mixture is filtered and the solid which is obtained is rinsed twice with 10 ml of acetic acid on each occasion, and the filtrate is then concentrated to dryness under reduced pressure. The residue which is obtained is taken up in 100 ml of distilled water, and this solution is concentrated to dryness under reduced pressure in order to yield a solid which is taken up in 50 ml of distilled water; this solution is then extracted with 3 times 50 ml of diethyl ether after 0.5 g of sodium sulphite have been added. The ether is separated off and the aqueous phase is placed under reduced pressure in order to eliminate traces of diethyl ether. 2 g of animal charcoal are added to the aqueous phase, which is heated at 40-50°C, and then filtered through Clarcel®; rinsing then takes place with a minimum of water. The pH is adjusted to 5 by adding 32% ammonia at 4°C. The precipitate which is obtained is filtered in the cold, rinsed with 2 times 10 ml of water, with 10 ml of ethanol and then with 2 times 10 ml of ether in order to yield, after drying under reduced pressure at 20°C, 3.97 g of (R,S)-4-isopropylphenylalanine in the form of a

white solid which melts at a temperature greater than 260°C. (See also Journal of the Takeda Research Laboratories, vol. 43; nos. 3/4, Dec. 1984, pp 53-76).

[0339] 36-2: 4-(Isopropylbenzylidene)-2-methyl-5-oxazolone

[0340] 18.52 g of N-acetylglycine, 10.6 g of sodium acetate, 20 ml of 4-isopropylbenzaldehyde and 57 ml of acetic anhydride are placed in a round-bottomed flask which is provided with a condenser. The mixture is stirred for 30 min and then stirred for 1 h at 110°C and subsequently for 15 h at room temperature. The reaction medium is poured into 600 ml of water and 400 ml of petroleum ether which has previously been heated to 50°C. The organic phase is separated off and the aqueous phase is washed with 2 times 150 ml of petroleum ether.

[0341] The organic phases are combined, dried over magnesium sulphate, filtered and concentrated under reduced pressure until the volume is 100 ml and a precipitate is obtained. The latter is filtered and washed with 2 times 50 ml of pentane in order to yield 8.2 g of 4-(isopropylbenzylidene)-2-methyl-5-oxazolone in the form of a yellow solid which melts at 77°C.

[0342] 36-3: (R,S)-4-Butylphenylalanine

[0343] 0.35 g of (R,S)-4-butylphenylalanine is obtained in the form of a light beige solid which melts at a temperature greater than 260° by proceeding as in Example 36-1 but using 1.49 g of red phosphorus, 1.8 g of 4-(butylbenzylidene)-2-methyl-5-oxazolone, in 9.23 ml of acetic anhydride, and 7.39 ml of 57% hydriodic acid.

[0344] 36-4: 4-(Butylbenzylidene)-2-methyl-5-oxazolone

[0345] 1.89 g of 4-(butylbenzylidene)-2-methyl-5-oxazolone are obtained in the form of a yellow solid which melts at 74°C by proceeding as in Example 36-2 but using 8.43 g of N-

acetylglycine, 4.92 g of sodium acetate, 9.8 g of 4-butylbenzaldehyde and 26 ml of acetic anhydride.

[0346] **EXAMPLE 37: Preparation of a derivative of phenylalanine using method D.**

[0347] 37-1: (R,S)-3-Ethoxyphenylalanine hydrochloride (or (R,S)-3-O-ethyltyrosine hydrochloride)

[0348] 1 g of (R,S)-N-tert-butoxycarbonyl-3-ethoxyphenylalanine, dissolved in 3.6 ml of hydrochloric dioxane, is placed in a round-bottomed flask, and the mixture is then stirred at room temperature for 5 h. The precipitate which forms is filtered, rinsed with dioxane and then ether, and then dried under reduced pressure (2.7 kPa) at 40°C to yield 0.65 g of (R,S)-3-ethoxyphenylalanine hydrochloride in the form of a white solid which melts at 200°C.

[0349] 37-2: (R,S)-N-tert-Butoxycarbonyl-3-ethoxyphenylalanine

[0350] 1.33 g of ethyl (R,S)-N-tert-butoxycarbonyl-3-ethoxyphenylalaninate, dissolved in 8 ml of methanol, are placed in a round-bottomed flask, and 8 ml of 1N sodium hydroxide solution are then added. After the mixture has been stirred at room temperature for 18 h, it is evaporated under reduced pressure and then acidified with 8.56 ml of 1N hydrochloric acid. The product is extracted with 2 times 10 ml of ethyl acetate, and the organic phases are pooled, washed with 2 times 10 ml of water, dried, filtered and then concentrated to dryness under reduced pressure to yield 1 g of (R,S)-N-tert-butoxycarbonyl-3-ethoxyphenylalanine in the form of a yellow oil (Merck Silica 5719, R_f=0.7, eluent: toluene 80/MeOH 10/diethylamine 10).

[0351] 37-3: (R,S)-N-tert-Butoxycarbonyl-3-ethoxyphenylalaninate

[0352] 1.5 g of (R,S)-N-tert-butoxycarbonyl-3-tyrosine, dissolved in 7.5 ml of dry DMF, are placed in a three-necked flask under a nitrogen atmosphere, and 0.508 g of sodium hydride,

as a 50% dispersion in oil, is then added. After the mixture has been stirred at room temperature for 2 h, 0.86 ml of iodoethane is added and the mixture is then stirred at room temperature for 4 h. The medium is filtered and the resulting solid is washed with 3 times 10 ml of water and then 2 times 10 ml of petroleum ether to yield, after drying under reduced pressure (2.7 kPa) at 30°C, 1.33 g of ethyl (R,S)-N-tert-butoxycarbonyl-3-ethoxyphenylalaninate in the form of a white solid.

[0353] 37-4: (R,S)-N-tert-Butoxycarbonyl-3-tyrosine

[0354] 18 g of (R,S)-3-tyrosine, dissolved in 180 ml of dioxane, are placed, with stirring, in a three-necked flask, and 99 ml of 1N sodium hydroxide solution, followed by 26 g of di-tert-butyl dicarbonate, dissolved in 160 ml of dioxane, are then added. After the mixture has been stirred for 36 h, it is concentrated under reduced pressure at 30°C and the residue is taken up in 100 ml of distilled water; this solution is acidified to pH 5 with 1N hydrochloric acid and then extracted with 2 times 200 ml of ethyl acetate. The organic phase is dried over magnesium sulphate, filtered and then concentrated to dryness under reduced pressure at 30°C to yield 30 g of (R,S)-N-tert-butoxycarbonyl-3-tyrosine in the form of a white solid (Merck Silica 5719, R_f=0.25, eluent: toluene 80, MeOH 10, diethylamine 10).

[0355] EXAMPLE 38: Preparation of derivatives of phenylalanine using method E.

[0356] 38-1: (RS)-4-Diallylaminophenylalanine dihydrochloride

[0357] A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 5.8 g of diethyl 4-diallylaminobenzylacetamido malonate and 48 ml of 10N hydrochloric acid; the solid is then triturated in 50 ml of acetone, filtered, then triturated in 10 ml of dichloromethane, filtered and then rinsed with 3 times 10 ml of ethyl ether. After drying under reduced pressure (2.7 kPa) at 40°C, 4.41 g of (RS)-4-diallylaminophenylalanine dihydrochloride

are obtained in the form of an off-white solid which melts in the region of 135°C (decomposition).

[0358] 38-2: (RS)-4-Allylaminophenylalanine dihydrochloride

[0359] A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 3.27 g of diethyl 4-allylaminobenzylacetamidomalonate and 30 ml of 10N hydrochloric acid; the solid is then triturated in 50 ml of acetone, filtered and then dried under reduced pressure (2.7 kPa) at 40°C. 2.3 g of (RS)-4-allylaminophenylalanine dihydrochloride are obtained in the form of a white solid which melts in the region of 134°C (decomposition).

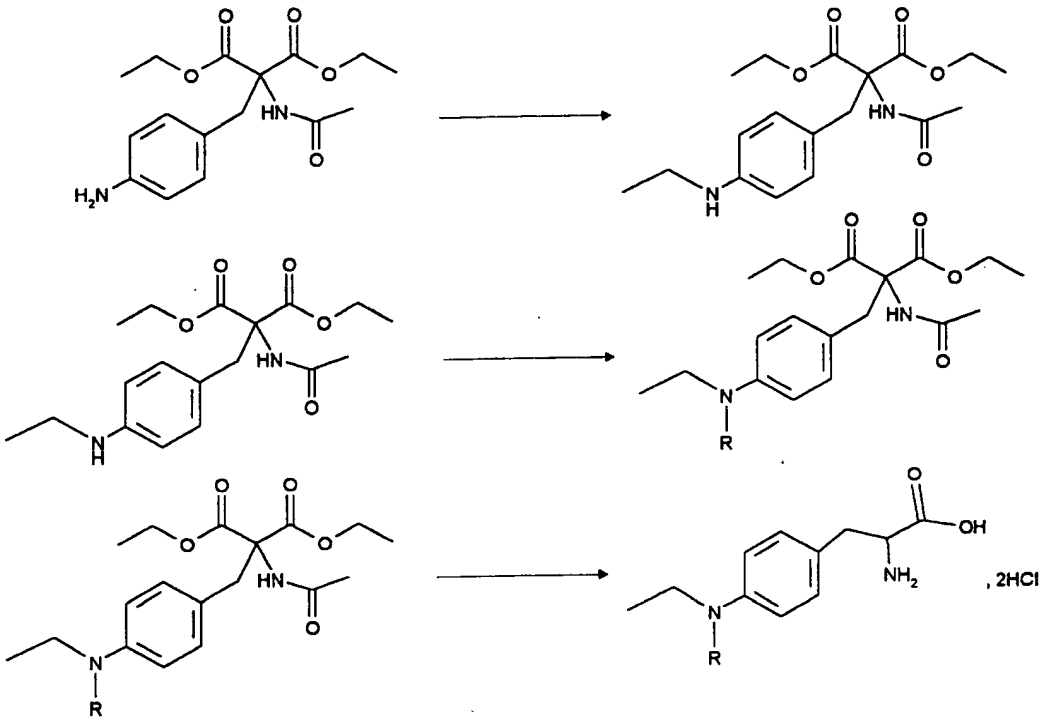
[0360] 38-3: Diethyl 4-diallylaminobenzylacetamidomalonate and diethyl 4-allylaminobenzylacetamidomalonate

[0361] 10 g of diethyl 4-aminobenzylacetamidomalonate dissolved in 150 ml of DMF are placed in a three-necked flask which is surmounted with a dropping funnel and maintained under a nitrogen atmosphere. 6.57 ml of allyl bromide, and then 10.76 ml of triethylamine, are added slowly, at room temperature and while stirring. After stirring for 19h, a further 1.31 ml of allylbromide and 2.15 ml of triethylamine are then added and the mixture is stirred for 26h. The reaction medium is poured onto 1.5 l of distilled water and this mixture is extracted with 1 l of ethyl acetate. The aqueous phase is separated off after settling and washed with 2 times 500 ml of ethyl acetate. The organic phases are combined, washed with 500 ml of distilled water and then with 500 ml of water which is saturated with sodium chloride, separated off, dried over magnesium sulphate, filtered and then concentrated to dryness in order to yield a chestnut oil; this oil is purified by flash chromatography (eluant, $CH_2Cl_2$90/AcOEt 10) in order to yield 6.66 g of diethyl 4-diallylaminobenzyl-acetamidomalonate in the form of a beige solid which melts at 94-96°C (Rf = 0.6, AcOEt 50/cyclohexane 50) and 3.49 g of diethyl 4-

allylaminobenzylacetamidomalonate in the form of a beige solid which melts at 104-106°C (Rf = 0.45 AcOEt 50/cyclohexane 50).

[0362] The diethyl 4-aminobenzylacetamidomalonate can be prepared as described in J.B. Burckhalter, VC Stephens, J. Am. Chem. Soc. 56, 1951, 73.

[0363] EXAMPLE 39: Preparation of derivatives of phenylalanine using method F



[0364] 39-1: (RS)-4-ethylisopropylphenylalanine dihydrochloride

[0365] A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 2.9 g of diethyl 4-ethylisopropylbenzylacetamidomalonate and 24.6 ml of 10N hydrochloric acid; the solid is then triturated in 20 ml of acetone, filtered and then dried under reduced pressure (2.7 kPa) at 40°C. 2 g of (RS)-4-ethylisopropylaminophenylalanine dihydrochloride are obtained in the form of a white solid which melts in the region of 147°C (decomposition).

[0366] 39-2: Diethyl 4-ethylisopropylaminobenzylacetamidomalonate

[0367] 15 g of diethyl 4-ethylaminobenzylacetamidomalonate in 70 ml of THF are placed in a three-necked flask which is maintained under a nitrogen atmosphere; 6.4 ml of 2-iodopropane, and then 8.4 ml of 1,5-diazabicyclo[4.3.0]non-5-ene are added and the mixture is then heated at 60°C for 24h. 2.13 ml of 2-iodopropane, and then 8.4 ml of 1,5-diazabicyclo[4.3.0]non-5-ene, are subsequently added and the mixture is then heated for a further 24h at 60°C. The mixture is brought to room temperature and then extracted with 50 ml of dichloromethane and 50 ml of distilled water. The aqueous phase is separated off after settling and then rewashed with 2 times 30 ml of dichloromethane. The organic phases are combined, washed with 60 ml of distilled water and then with 50 ml of distilled water which is saturated with NaCl. The organic phase is separated off after settling, dried over magnesium sulphate, filtered and then concentrated to dryness under reduced pressure in order to yield 16.2 g of a product which is purified by flash chromatography (dichloromethane, MeOH 90/10). This results in 4.59 g of a product which is recrystallized in 45 ml of cyclohexane in order to yield 3.44 g of diethyl 4-ethylisopropylamino-benzylacetamidomalonate in the form of white crystals which melt at 80°C.

[0368] 39-3: Diethyl 4-ethylaminobenzylacetamidomalonate

[0369] Diethyl 4-ethylaminobenzylacetamidomalonate can be prepared by proceeding as in Example 35-7 but using 22 g of diethyl 4-aminobenzylacetamidomalonate, 500 ml of ethanol and 70 g of Raney nickel. This results in 23.8 g of diethyl 4-ethylaminobenzylacetamidomalonate in the form of an off-white solid which melts at 136°C.

[0370] 39-4: (RS)-4-Allylethylaminophenylalanine dihydrochloride

[0371] A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 4.54 g of diethyl 4-allylethylbenzylacetamidomalonate and 37.9 ml of 10N hydrochloric acid; the

solid is then dried under reduced pressure (2.7 kPa) at 40°C. 3.67 g of (RS)-4-allylethylaminophenylalanine dihydrochloride are obtained in the form of a brown solid which melts in the region of 130°C (decomposition).

[0372] 39-5: Diethyl 4-allylethylaminobenzylacetamidomalonate

[0373] 5.6 g of a solid are obtained, after purification by flash chromatography (eluant, CH2Cl2/AcOET 90-10 by volume), by proceeding as in Example 39-2 but using 8 g of diethyl 4-ethylaminobenzylacetamidomalonate, 4 ml of allyl bromide and 5.82 ml of 1,5-diazabicyclo[4.3.0]non-5-ene in 50 ml of THF; the solid is then recrystallized in 35 ml of cyclohexane. This results in 5.43 g of diethyl 4-allylethylaminobenzylacetamidomalonate in the form of a white solid which melts at 86°C.

[0374] 39-6: (RS)-4-Ethylpropylaminophenylalanine dihydrochloride

[0375] A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 2.5 g of diethyl 4-ethylpropylaminobenzylacetamidomalonate and 21 ml of 10N hydrochloric acid;. The solid is then dried under reduced pressure (2.7 kPa) at 40°C. 2 g (97%) of (RS)-4-ethylpropylaminophenylalanine dihydrochloride are obtained in the form of a white solid which melts in the region of 147°C (decomposition).

[0376] 39-7: Diethyl 4-ethylpropylaminobenzylacetamidomalonate

[0377] 2.8 g of a solid are obtained, after reacting for 36 hours and then purifying by flash chromatography (eluant, CH2Cl2/MeOH 97-3 by volume), by proceeding as in Example 39-2 but using 10 g of diethyl 4-ethylaminobenzylacetamidomalonate, 5.6 ml of 1-iodopropane and 7.2 ml of 1,5-diazabicyclo[4.3.0]non-5-ene in 70 ml of THF; the solid is then recrystallized in 26 ml of cyclohexane. This results in 2.9 g of diethyl 4-ethylpropylaminobenzylacetamidomalonate in the form of a white solid which melts at 84-86°C.

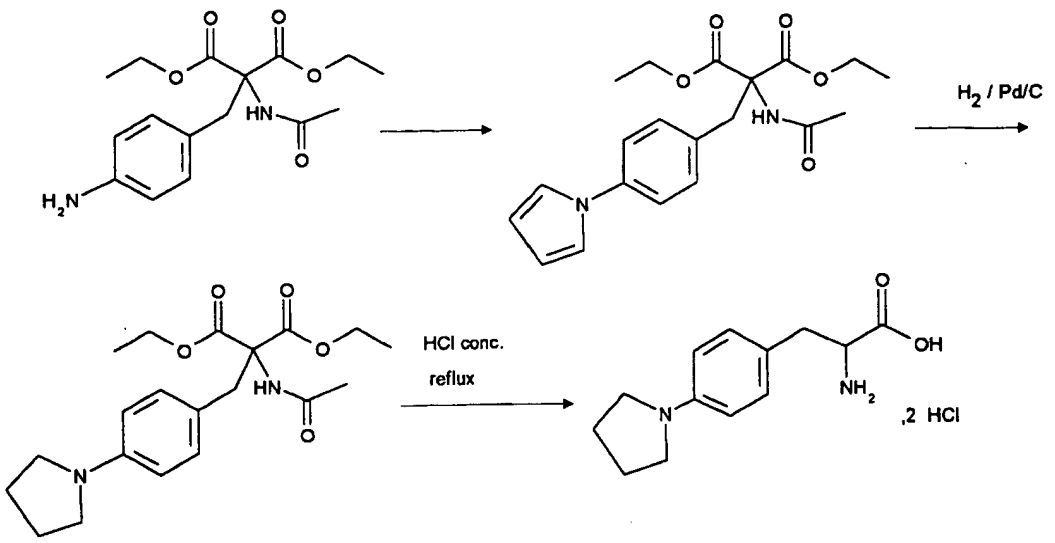
[0378] 39-8: (RS)-4-Ethylmethylcyclopropylaminophenylalanine dihydrochloride

[0379] A solid is obtained, after reacting for 3 days and then evaporating, by proceeding as in Example 35-1 but using 3 g of diethyl 4-ethylmethylcyclopropylaminobenzylacetamidomalonate and 25 ml of 10N hydrochloric acid; the solid is then triturated in 40 ml of acetone, filtered and then dried under reduced pressure (2.7 kPa) at 40°C. 2.24 g of (RS)-4-ethylmethylcyclopropylamino-phenylalanine dihydrochloride are obtained in the form of a white solid which melts in the region of 140°C (decomposition).

[0380] 39-9: Diethyl 4-ethylmethylcyclopropylaminobenzylacetamidomalonate

[0381] By proceeding as in Example 39-2, but using 8 g of diethyl 4-ethylaminobenzylacetamidomalonate, 2.6 ml of bromomethylcyclopropane and 2.97 ml of 1,5-diazabicyclo[4.3.0]non-5-ene in 50 ml of THF, 3.3 g of diethyl 4-ethylmethylcyclopropylaminobenzylacetamidomalonate are obtained, after reacting for 3 days and then purifying by flash chromatography (eluant CH_2Cl_2/AcOEt 90-10 by volume), in the form of a white solid which melts at 112-114°C.

[0382] EXAMPLE 40: Preparation of derivatives of phenylalanine using method G



[0383] 40-1: (RS)-4-(1-Pyrrolidinyl)phenylalanine dihydrochloride

[0384] A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 1.5 g of diethyl 4-(1-pyrrolidinyl)benzylacetamidomalonate and 40 ml of 5N hydrochloric acid; the solid is then triturated in 15 ml of acetone, filtered and then dried under reduced pressure (2.7 kPa) at 40°C. 0.6 g of (RS)-4-(1-pyrrolidinyl)phenylanaline dihydrochloride is obtained in the form of an off-white solid.

[0385] 40-2: Diethyl 4-(1-pyrrolidinyl)benzylacetamidomalonate

[0386] 4 g of diethyl 4-(1-pyrrolyl)benzylacetamidomalonate, dissolved in 100 ml of MeOH, and 1 g of 10% palladium on charcoal are placed in an autoclave. After having purged the autoclave 3 times with nitrogen, the product is hydrogenated at 19°C under a pressure of 14 bars of hydrogen. After stirring for 25 hours, the hydrogenation is stopped and the product is filtered through Clarcel® and rinsed with dichloromethane; the solution is then concentrated under reduced pressure in order to yield 3.85 g of a solid which is triturated in a mixture of 50 ml of heptane and 10 ml of ethyl ether. The resulting solid is filtered, dried and then purified by

flash chromatography (eluant CH_2Cl_2/acetone 90/10 by volume) in order to yield 1.6 g of diethyl 4-(1-pyrrolidinyl)benzylacetamidomalonate in the form of a white solid which melts at 132°C.

[0387] 40-3: Diethyl 4-(1-pyrrolyl)benzylacetamidomalonate

[0388] 4,6 g of diethyl 4-aminobenzylacetamidomalonate in 104 ml of acetic acid are placed in a three-necked flask which is maintained under nitrogen. 7.02 g of sodium acetate are added, followed by 1.87 ml of 2,5-dimethoxytetrahydrofuran. The mixture is heated at 65°C for 1h 15 min, then cooled down and extracted with 100 ml of dichloromethane and 100 ml of distilled water. The aqueous phase is separated off after settling and then washed with 3 times 100 ml of dichloromethane. The organic phases are combined, washed with 100 ml of water and then with 100 ml of a saturated solution of NaCl, separated off after settling and then dried over magnesium sulphate; the phases are filtered and then evaporated to dryness under reduced pressure (50 kPa) in order to yield 6.2 g of a solid which is purifed by flash chromatography (eluent CH2Cl2/acetone 75/25 by volume). This results in 3.57 g of diethyl 4-(1-pyrrolyl)benzylacetamidomalonate in the form of a beige solid which melts at 110°C.

[0389] EXAMPLE 41: Preparation of derivatives of phenylalanine using method H

[0390] 41-1: (RS)-4-Ethylthiomethylphenylalanine

[0391] 300 ml of anhydrous methanol are placed in a three-necked flask which is maintained under nitrogen; subsequently, 1.72 g of sodium methoxide, and then 5.55 ml of ethyl mercaptan, are added while stirring. The solvent is concentrated under reduced pressure at 40°C in order to yield 8.5 g of the sodium salt of ethyl mercaptan, which is dissolved in 100 ml of anhydrous THF. 3.6 g of (RS)-4-chloromethylphenylalanine are added at room temperature and the mixture is then heated to reflux for 18h. The solvent is evaporated under reduced pressure at 40°C and the residue is taken up in 100 ml of distilled water. The turbid solution which is

obtained is acidified with 5 ml of acetic acid. The resulting precipitate is filtered, rinsed with distilled water and then dried at 60)C under reduced pressure in order to yield 3.6 g of a solid which is purified by flash chromatography (eluant AcOEt 60, AcOH 12, water 10). This results in 256 mg of (RS)-4-ethylthiomethylphenylalanine in the form of a white solid which melts at 251°C.

[0392] The (RS)-4-chloromethylphenylalanine can be obtained by analogy with (S)-4-chloromethylphenylalanine as described in: R.Gonzalez-Muniz, F. Cornille, F. Bergeron, D. Ficheux, J. Pothier, C. Durieux and B. Roques, Int. J. Pept. Protein. Res., 1991, 37 (41), 331-340.

[0393] EXAMPLE 42: Preparation of derivatives of phenylalanine using method I

[0394] 42-1: (S)-4-O-(2-Chloroethyl)tyrosine hydrochloride

[0395] 5 g of (S)-N-tert-butoxycarbonyl-4-O-(2-chloroethyl)tyrosine, dissolved in 50 ml of hydrochloric dioxane, are placed in a round-bottomed flask. After having been stirred for 28h, the mixture is concentrated to dryness under reduced pressure. The resulting residue is taken up in 50 ml of ether and this solution is then stirred and filtered. The resulting solid is washed with 2 times 25 ml of ether and then dried under reduced pressure in order to yield 1.58 g of (S)-4-O-(2-chloroethyl)tyrosine hydrochloride in the form of a white solid which melts at 260°C.

[0396] 42-2: (S)-N-tert-Butoxycarbonyl-4-O-(2-chloroethyl)tyrosine

[0397] 14 g of (S)-N-tert-butoxycarbonyltyrosine, dissolved in 140 ml of DMF, are placed in a three-necked flask under a nitrogen atmosphere. 4.8 g of 50% sodium hydride in oil are added slowly using a spatula. 16.87 g of 1-tosyl-2-chloroethanol are added after the mixture has been stirred for 2h at room temperature. 2.4 g of 50% sodium hydride in oil, and a further 8.4 ml of 1-tosyl-2-chloroethanol, are added after the mixture has been stirred for 2 days. The same procedure is carried out after 24h and the stirring is continued for a further 24h. The

reaction is stopped by adding 100 ml of distilled water, and the reaction mixture is concentrated to dryness under reduced pressure. The residue which is obtained is taken up in 100 ml of distilled water and then extracted with 3 times 100 ml of ethyl acetate. The aqueous phase is separated off after settling and acidified to pH3 with 50 ml of 1N HCl, and the product is extracted with 3 times 100 ml of ethyl acetate. The organic phases are combined, washed with 2 times 50 ml of water, separated off, dried over magnesium sulphate, filtered and then concentrated to dryness under reduced pressure in order to yield 13.51 g of (S)-N-tert-butoxycarbonyl-4-O-(2-chloroethyl)tyrosine in the form of a chestnut oil (Rf = 0.5, toluene 70%/methanol 20%/diethylamine 10%), which is used as such in the following step.

[0398] TABLE V

MICROORGANISMS	ANTIBIOTICS
FUNGI	
Micromonospora sp.	Vernamycins
STREPTOMYCES	
S. alborectus	Virginiamycins
S. conganensis (ATCC13528)	F1370 A, B
S. diastaticus	Plauracins, Streptogramins
S. graminofasciens	Streptogramins
S. griseus (NRRL2426)	Viridogrisein (Etamycin)
S. griseoviridus	Griseoviridin
S. griseoviridus (FERMP3562)	Neoviridogriseins
S. lavendulae	Etamycins
S. loidensis (ATCC11415)	Vernamycins
S. mitakaensis (ATCC15297)	Mikamycins
S. olivaceus (ATCC12019)	Synergistins (PA 114 A, B)
S. ostreogriseus (ATCC27455)	Ostreogrycins
S. pristinaespiralis (ATCC25486)	Pristinamycins
S. virginiae (ATCC13161)	Virginiamycins (Staphylomycins)
ACTINOMYCETES	

A. auranticolor (ATCC31011)	Plauracins
A. azureus (ATCC31157)	Plauracins
A. daghestanicus	Etamycin
A. philippinensis	A-2315 A,B,C
Actinoplanes sp. (ATCC3302)	A15104
Actinoplanes sp.	A17002 A,B,C,F
Actinomadura flava	Madumycins

[0399] Abbreviations employed:

AcOEt	ethyl acetate
DNA	deoxyribonucleic acid
AMP	adenosine 5'-monophosphate
HPLC	high-performance liquid chromatography
dCTP	deoxycytosine 5'-triphosphate
DMF	dimethylformamide
DMPAPA	4-dimethylamino-L-phenylalanine
HCl	hydrochloric acid
HT7	Hickey Tresner solid medium
3-HPA	3-hydroxypicolinic acid
IPTG	isopropyl-β-D-thiogalactopyranoside
kb	kilobase
LB	Luria broth (rich growth medium for E. coli)
MeOH	methanol
MMPAPA	4-methylamino-L-phenylalanine
NaOH	sodium hydroxide
PAPA	4-amino-L-phenylalanine

PEG	polyethylene glycol
P I	pristinamycin I
P II	pristinamycin II
bp	base pair
SAM	S-adenosylmethionine
TE	10 mM Tris-HCl buffer, 1 mM EDTA, pH 7.5
THF	tetrahydrofuran
Tris	2-amino-2-(hydroxymethyl)-1,3-propanediol
UV	ultraviolet rays
X-gal	5-bromo-4-chloro-3-indoyl-β-D-galactoside
YEME	yeast extract-malt extract medium (rich growth medium for Streptomyces)

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